

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

BIO-RAD LABORATORIES, INC. and
THE UNIVERSITY OF CHICAGO,

Plaintiffs,

v.

10X GENOMICS, INC.,

Defendant.

)
)
)
)
)
)
)
)
)
)
)

C.A. No. 15-152-RGA

FILED UNDER SEAL

**EXHIBITS A-L TO PLAINTIFF'S SEPTEMBER 1, 2017
DISCOVERY DISPUTE LETTER (D.I. 215)**

Dated September 1, 2017

Edward R. Reines (admitted *pro hac vice*)
Derek Walter (admitted *pro hac vice*)
WEIL, GOTSHAL & MANGES LLP
201 Redwood Shores Parkway
Redwood Shores, CA 94065
(650) 802-3000

Joseph J. Farnan, Jr. (Bar No. 100245)
Brian E. Farnan (Bar No. 4089)
Michael J. Farnan (Bar No. 5165)
FARNAN LLP
919 N. Market St., 12th Floor
Wilmington, DE 19801
Tel: (302) 777-0300
Fax: (302) 777-0301
farnan@farnanlaw.com
bfarnan@farnanlaw.com
mfarnan@farnanlaw.com

*Attorneys for Plaintiffs
BIO-RAD LABORATORIES, INC. and THE
UNIVERSITY OF CHICAGO*

EXHIBIT A

Trials@uspto.gov
571-272-7822

Paper 51
Entered: November 15, 2016

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC.,
Petitioner,

v.

THE UNIVERSITY OF CHICAGO,
Patent Owner.

Case IPR2015-01157
Patent 8,889,083 B2

Before DONNA M. PRAISS, CHRISTOPHER L. CRUMBLEY, and
TINA E. HULSE, *Administrative Patent Judges*.

CRUMBLEY, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
35 U.S.C. § 318 and 37 C.F.R. § 42.73

IPR2015-01157

Patent 8,889,083 B2

I. INTRODUCTION

In this *inter partes* review trial, instituted pursuant to 35 U.S.C. § 314, Petitioner 10X Genomics, Inc. (“10X”) challenges the patentability of claims 1–31 of U.S. Patent No. 8,889,083 B2 (“the ’083 patent,” Ex. 1001), owned by The University of Chicago (“Chicago”).

We have jurisdiction under 35 U.S.C. § 6(b). This Final Written Decision, issued pursuant to 35 U.S.C. § 318(a), addresses issues and arguments raised during trial. For the reasons discussed below, we determine that 10X has not proven, by a preponderance of the evidence, that claims 1–31 of the ’083 patent are unpatentable.

A. Procedural History

On May 6, 2015, 10X requested an *inter partes* review of claims 1–31 of the ’083 patent. Paper 1, “Pet.” Chicago filed a Patent Owner Preliminary Response. Paper 9, “Prelim. Resp.” In a Decision on Institution of *Inter Partes* Review (Paper 14, “Dec. on Inst.”), we instituted trial as to claims 1–31 on the following grounds of unpatentability:

1. Whether claims 1–31 are unpatentable under 35 U.S.C. § 103(a) as having been obvious over Quake¹ and Ramsey;²
2. Whether claims 18 and 24 are unpatentable under 35 U.S.C. § 103(a) as having been obvious over Quake, Ramsey, and Green.³

Dec. on Inst. 21.

¹ Quake et al., US 2002/0058332 A1, published May 16, 2002 (Ex. 1004).

² Ramsey et al., US 6,524,456 B1, issued Feb. 25, 2003 (Ex. 1006).

³ Green, S.W. et al., *Perfluorocarbon Fluids*, Organofluorine Chemistry: Principles and Commercial Applications (1994) (Ex. 1007).

IPR2015-01157

Patent 8,889,083 B2

Following institution of trial, Chicago filed a Patent Owner Response (Paper 19, “PO Resp.”), and 10X filed a Reply (Paper 25, “Pet. Reply”).

10X supported its Petition with the Declaration of Wilhelm T.S. Huck, Ph.D. Ex. 1002. Chicago took cross-examination testimony of Dr. Huck via deposition on January 7–8, 2016, and submitted the transcript of that deposition. Exs. 2026, 2027.

With its Response, Chicago submitted the Declaration of Samuel Sia, Ph.D. Ex. 2028. 10X cross-examined Dr. Sia via deposition on March 25, 2016, and submitted the transcript. Ex. 1073.

With its Reply, 10X submitted a Second Declaration of Dr. Huck (Ex. 1087), and submitted the declaration testimony of a second witness, Peter David Olmsted, Ph.D. (Ex. 1085). Chicago took cross-examination testimony via deposition of Dr. Huck on May 17, 2016, and of Dr. Olmsted on May 13, 2016, and submitted the transcripts to the Board. Exs. 2051 (Olmsted), 2052 (Huck).

Chicago filed Observations on the Cross-Examination of Drs. Olmsted and Huck (Paper 35), and 10X filed a Response (Paper 39). As authorized by the Board (Paper 30), Chicago also filed an Identification of Testimony from Dr. Huck that allegedly exceeds the proper scope of reply (Paper 34), and 10X filed a Response to that list (Paper 38).

Chicago filed a Motion to Exclude various exhibits submitted by 10X (Paper 36, “Mot.”), to which 10X filed an Opposition (Paper 40) and Chicago filed a Reply (Paper 43).

IPR2015-01157

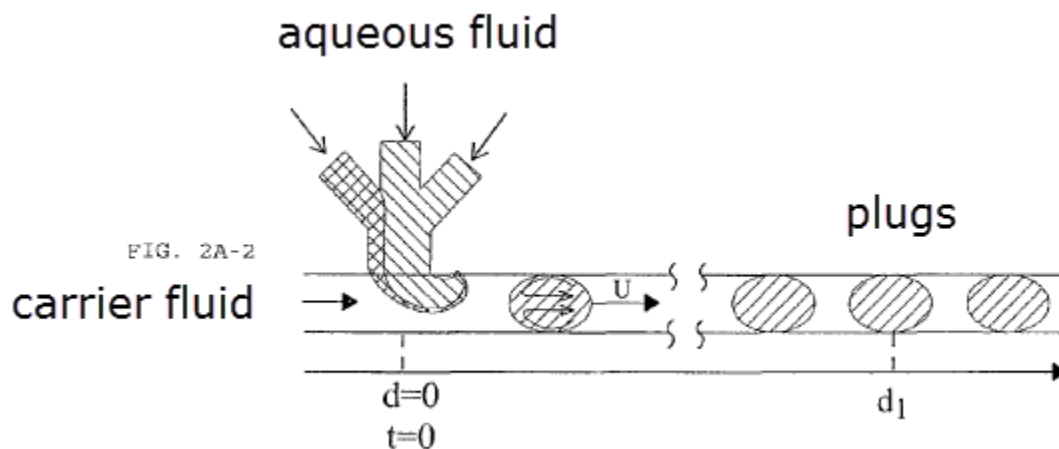
Patent 8,889,083 B2

Oral hearing was requested by both parties (Papers 31, 33), and argument before the Board was held June 30, 2016. A transcript of the oral hearing is included in the record. Paper 50, “Tr.”

B. The '083 Patent

The '083 patent, entitled “Device and Method for Pressure-Driven Plug Transport and Reaction,” issued November 18, 2014. Ex. 1001, (45), (54). Microfluidic systems transport fluids through networks of channels, typically having micrometer dimensions. *Id.* at 1:18–20. According to the specification, the main advantages of microfluidic systems are high speed and low consumption of reagents. *Id.* at 1:23–25. The microfluidic systems may be used for various chemical and biochemical processes, including autocatalytic reactions, such as the polymerase chain reaction (PCR). *Id.* at 44:38–61.

Rather than rely on laminar flow of liquids through microfluidic channels, the '083 patent specification describes using microfluidic droplets, or “plugs” in the system. Figure 2A-2, an embodiment of the system, is annotated and reproduced below:



IPR2015-01157

Patent 8,889,083 B2

Figure 2A-2 depicts the formation of plugs, where the aqueous fluid containing a substrate and reagents enters a channel with an immiscible carrier fluid, such as an oil, to form a series of plugs. *Id.* at 17:17–23.

In one disclosed embodiment, the walls of the channels are not fluorinated, while the carrier fluid is a fluorinated oil that contains a fluorinated surfactant. *Id.* at 20:63–21:2. According to the specification, “[b]ecause the walls of the channels (PDMS, not fluorinated) and the carrier-fluid (fluorinated oil) are substantially different chemically, when a fluorinated surfactant is introduced, the surfactant reduces the surface tension at the oil-water interface preferentially over the wall-water interface.” *Id.* This difference in surface tension results in the carrier fluid preferably wetting the walls of the channels, as opposed to wetting the plugs, meaning that the plugs typically do not contact the walls of the channel. *Id.* at 20:41–45. “This allows the formation of plugs that do not stick to the channel walls.” *Id.* at 21:1–2.

C. Illustrative Claim

Of the challenged claims, 1, 20, and 31 are independent, with claims 1 and 31 directed to microfluidic systems and claim 20 directed to a method of conducting a reaction within a plug in a microfluidic system. Claim 1 is illustrative and is reproduced below:

1. A microfluidic system comprising:
 - a non-fluorinated microchannel;
 - a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel;
 - at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid,

IPR2015-01157

Patent 8,889,083 B2

wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.

Ex. 1001, 73:11–21.

II. ANALYSIS

A. Claim Construction

For purposes of our Decision on Institution, we analyzed each claim term in light of its broadest reasonable interpretation, as understood by one of ordinary skill in the art and consistent with the specification of the '083 patent. 37 C.F.R. § 42.100(b); *Cuozzo Speed Techs., LLC v. Lee*, 136 S. Ct. 2131, 2144–46 (2016). Under the broadest reasonable interpretation standard, and absent any special definitions, claim terms are given their ordinary and customary meaning, as would be understood by one of ordinary skill in the art in the context of the entire disclosure. *In re Translogic Tech. Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007). Any special definitions for claim terms or phrases must be set forth with reasonable clarity, deliberateness, and precision. *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

In the Decision on Institution, we evaluated the parties' proffered constructions for the claim terms "at least one plug comprising an aqueous plug fluid" (claims 1 and 31) and "at least one plug." Dec. on Inst. 7–8. We determined that, based on the record at the time, the broadest reasonable interpretation is "at least one volume of aqueous fluid formed when a stream of aqueous fluid is introduced into the flow of a substantially immiscible carrier-fluid." *Id.* at 8. We also evaluated the other constructions proffered

IPR2015-01157

Patent 8,889,083 B2

by the parties,⁴ and determined that the remaining terms did not require express construction. *Id.* at 8–10. During the instituted trial, neither party disputed our preliminary claim constructions as set forth in the institution Decision or raised additional claim construction arguments. Upon review of the entire record, we maintain our prior constructions.

B. Obviousness over Quake and Ramsey

10X contends that claims 1–31 are unpatentable under 35 U.S.C. § 103(a), as they would have been obvious over the combined disclosures of Quake and Ramsey. Pet. 18–50. An obviousness inquiry involves four underlying determinations: the scope and content of the prior art; the differences between the prior art and the claims at issue; the level of ordinary skill; and any objective indicia of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

Quake relates to microfluidic devices and methods for analyzing and/or sorting biological materials that “offer several advantages over traditional flow cytometry devices and methods.” Ex. 1004, Abstract, ¶ 14. The devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. *Id.* ¶ 3. The devices comprise a main channel, through which a pressurized stream of oil is

⁴ Specifically, both parties proffered constructions for “fluorinated surfactant comprising a hydrophilic head group” and “surface tension at the plug-fluid/microchannel wall interface is higher than the surface tension at the plug-fluid/carrier fluid interface.” Pet. 13–15; Prelim. Resp. 22–30. 10X proffered a construction for two additional claim terms: “non-fluorinated microchannel” (Pet. 10–13) and “substantially encased/substantially surrounded on all sides” (*id.* at 15–17).

IPR2015-01157

Patent 8,889,083 B2

passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* The channels of the device may be formed from a silicon elastomer, including polydimethylsiloxane (PDMS), or urethane. *Id.* ¶¶ 118, 216.

A junction joins the main channel with the sample inlet channel. *Id.* ¶ 3. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

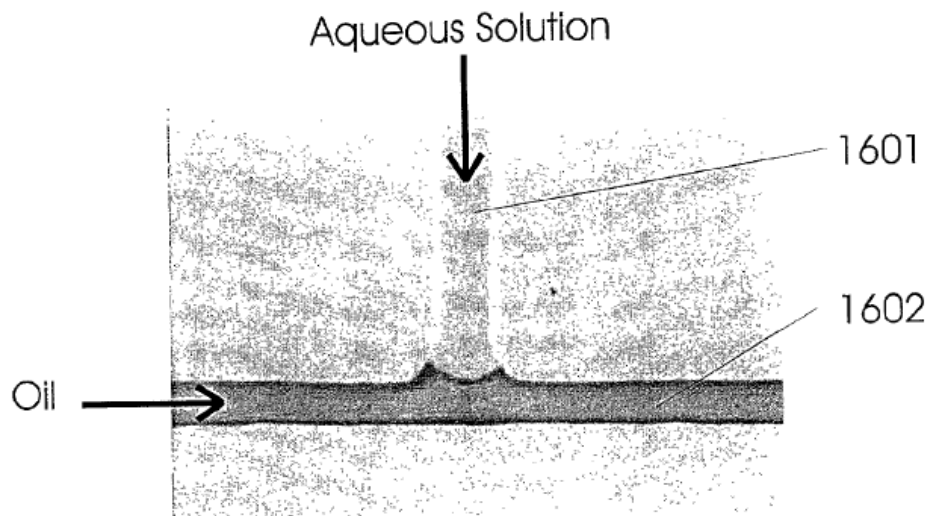


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region.

Channel 1601 containing an aqueous solution intersects with the main channel 1602 containing oil. *Id.* ¶ 292.

Quake discloses that the fluid that forms the droplet is typically an aqueous buffer solution that is physiologically compatible with the molecules in the

IPR2015-01157

Patent 8,889,083 B2

droplet. *Id.* ¶ 116. The carrier fluid⁵ in which the droplets are formed is a fluid that is not miscible with the droplet fluid, preferably a nonpolar solvent such as decane or another oil. *Id.* The fluids may contain additives, such as surfactants, which Quake teaches “may aid in controlling or optimizing droplet size, flow and uniformity.” *Id.* ¶ 117. Quake discloses that the surfactant may be contained in the carrier fluid, such that when the fluid flows through the channels of the microfluidic device, the surfactant coats the channel walls. *Id.* ¶ 96. Such a coating minimizes adhesion, preventing material such as “cells, virions and other particles or molecules” from adhering to the sides of the channels. *Id.* ¶ 94.

Ramsey relates to “a microfabricated channel device that can manipulate nanoliter or subnanoliter biochemical reaction volumes in a controlled manner to produce results at rates of 1 to 10 Hz per channel.” Ex. 1006, Abstract. Ramsey discloses separating volume segments using a segmenting material that “is preferably a liquid that is immiscible in . . . the reaction fluid(s).” *Id.* at 3:63–67, 6:36–37. Ramsey also discloses that “the segmenting fluid should be biocompatible with [the] biological reagents that are used” in the reaction fluid and, specifically, that “[p]erfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:37–39, 49–50.

⁵ Quake uses the term “extrusion” or “barrier” fluid to refer to the fluid that surrounds the droplets (Ex. 1004 ¶ 20); we use the terminology of the ’083 patent.

IPR2015-01157

Patent 8,889,083 B2

1. Content of the Record

Before turning to the merits of the parties' arguments, we address Chicago's contention that the Second Declaration of Dr. Huck (Ex. 1087) exceeds the proper scope of reply. As summarized in our May 26, 2016 Order, Chicago requested authorization to file a motion to strike Dr. Huck's Second Declaration as improper reply testimony. Paper 30, 2. We declined to authorize a motion to strike, because the Board is capable of determining at the close of evidence whether new arguments were raised and disregarding any improper reply. We permitted Chicago to file a short list setting forth the allegedly improper testimony. *Id.* Chicago filed its list (Paper 34), and 10X filed a responsive paper (Paper 38).

"It is of the utmost importance that petitioners in [*inter partes* review] proceedings adhere to the requirement that the initial petition identify 'with particularity' the 'evidence that supports the grounds for the challenge to each claim.'" *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1369 (Fed. Cir. 2016) (quoting 35 U.S.C. § 312(a)(3)). For this reason, our Rules require that "[a] reply may only respond to arguments raised in the corresponding opposition . . . or patent owner response." *See* 37 C.F.R. § 42.23(b). This reasoning applies equally to reply declarations, submitted to support a party's reply brief. *See Intelligent Bio-Sys., Inc.*, 821 F.3d at 1369–70 (affirming exclusion of reply brief and supporting declaration). Our Trial Practice Guide provides that "a reply that raises a new issue or belatedly presents evidence will not be considered. . . . The Board will not attempt to sort proper from improper portions of a reply."

IPR2015-01157

Patent 8,889,083 B2

Office Patent Trial Practice Guide, 77 Fed. Reg. 48,756, 48,767 (Aug. 14, 2012) (“Trial Practice Guide”).

Of course, “the introduction of new evidence in the course of the trial is to be expected in *inter partes* review trial proceedings.” *Genzyme Therapeutic Prod. Ltd. P’ship v. Biomarin Pharm. Inc.*, 825 F.3d 1360, 1366 (Fed. Cir. 2016). That new evidence, however, must be responsive to an argument raised by the opposing party in its opposition brief. By contrast, when “new evidence necessary to make out a prima facie case for . . . unpatentability” or “new evidence that could have been presented in a prior filing” is introduced, these are indications that a new issue has been raised improperly. *See* Trial Practice Guide, 77 Fed. Reg. at 48,767.

Chicago identifies four general topics of testimony, and supporting exhibits, that allegedly exceed the proper scope of reply. We address each in turn below.

a. Testimony on State of the Art of Microfluidics

First, Chicago challenges ¶¶ 24–39 of Dr. Huck’s Second Declaration, pertaining to various “microfluidics conferences, journals, companies, and products.” Paper 34, 1. Several exhibits supporting this testimony are also identified: Exs. 1041–1045, 1047, 1051–1056, 1068, 1071–1072, 1081–1082, and 1088–1092. *Id.* at 1–2. In this portion of his testimony, Dr. Huck is testifying regarding the state of the art of microfluidics, and supporting this testimony with citations to various conferences, journals, and the like. 10X contends that this testimony is responsive to “Dr. Sia’s allegation that microfluidics was a nascent field in 2002.” Paper 38, 1.

IPR2015-01157

Patent 8,889,083 B2

Upon review, we determine this testimony and the supporting documents to be proper reply evidence. In his Declaration, Dr. Sia testifies that there was a “limited amount of research in this area,” and disputes the familiarity that the skilled artisan would have with various microfluidics techniques. Ex. 2028 ¶ 30. 10X was entitled to introduce evidence responsive to this testimony. Furthermore, we note that Dr. Huck’s testimony and the supporting documents are not being offered to establish 10X’s proposed ground of unpatentability, but rather to provide general background information for establishing the state of the art. *See Genzyme*, 825 F.3d at 1367 (permitting reply evidence “relevant to show the state of the art at the time of the inventions”).

b. Testimony on Knowledge of Reactions in Droplets

Chicago also objects to ¶¶ 104–111 of Dr. Huck’s Second Declaration, along with supporting Exhibits 1049–1050, 1059, 1078, and 1093. Paper 34, 1–2. This testimony and accompanying exhibits pertain to performing reactions in microfluidic droplets, particularly PCR in droplets. 10X argues that this testimony is responsive to Dr. Sia’s testimony that a person of ordinary skill would have had limited knowledge of droplet PCR. Paper 38, 2.

This presents a closer question, because the knowledge of PCR in droplets—particularly, the expectation of success in performing PCR in droplets—is relevant to the ground of unpatentability as to claims 11 and 21, which expressly require performance of PCR in the droplets. The Petition, however, relies on Quake itself for this reasonable expectation of success. Pet. 45–46. Dr. Sia addresses the cited portion of Quake in his testimony,

IPR2015-01157

Patent 8,889,083 B2

contending that a “host of other problems and complications left unaddressed by both Quake and Ramsey would have prevented the skilled artisan from having a reasonable expectation of successfully being able to carry out PCR or other autocatalytic reactions in microfluidic droplets,” and goes on to provide examples of these issues. Ex. 2028 ¶ 105. After reviewing the testimony of Dr. Huck, we determine that it properly replies to this testimony by Dr. Sia, and cites the supporting exhibits to dispute these alleged “problems and complications.”

c. Testimony as to Paragraph 117 of Quake

Chicago objects to ¶¶ 45–48 and 71–74 of the Second Huck Declaration, which consist of testimony regarding ¶ 117 of Quake. Paper 34, 1. This testimony is closely tied to an allegedly new argument raised in 10X’s Reply, which we address in the following section. For the reasons discussed below, we determine that Dr. Huck’s testimony in these portions exceeds the proper scope of reply.

d. Testimony as to Routine Optimization

Finally, Chicago challenges ¶¶ 76–78 of Dr. Huck’s Second Declaration, which explain Dr. Huck’s assertion in ¶ 75 that “selecting the surfactant of choice and the amount of surfactant would have been a matter of routine optimization.” After reviewing the testimony, we determine that this portion raises a new issue that alters the basis of Dr. Huck’s testimony, and thus exceeds the proper scope of reply.

Dr. Huck’s first Declaration does not rely on routine optimization of the surfactant concentration to achieve the claimed surface tension

IPR2015-01157

Patent 8,889,083 B2

relationship (“surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface”).⁶ *See generally* Ex. 1002 ¶¶ 59–63. Rather, Dr. Huck contends that—in the terminology of the ’083 patent—the surface tension relationship “merely represents a condition achieved when sufficient fluorinated surfactant is present in the carrier fluid such that the plugs do not adhere to the channel wall.” *Id.* ¶ 44. Dr. Huck then testifies that because Quake discloses surfactants that coat the walls of its channels, a person of ordinary skill in the art “would have understood that Quake discloses the use of a fluorinated surfactant in the carrier fluid to prevent the plugs from adhering to the microchannel wall.” *Id.* ¶ 61. As a result, Dr. Huck concludes, the person of ordinary skill in the art “would have understood that the combination of Quake and Ramsey discloses a microfluidics system where the surface tension” relationship is present. *Id.*

From this testimony, it is clear that Dr. Huck’s theory in his first Declaration was that the behavior of Quake’s droplets signifies that the surface tension relationship—and, therefore, sufficient surfactant—was *present* in the system. By contrast, routine optimization relies on a *modification* of the disclosed system to achieve a surfactant concentration that is not disclosed by the reference. *See* Ex. 1087 ¶ 76 (“Quake teaches a range of suitable surfactant concentrations from between 0.2 and 5%. . . . Following Quake’s teaching of adding surfactant to the carrier oil, a [person of ordinary skill in the art] would have routinely optimized the amount of

⁶ During trial, the parties adopted the shorthand of referring to this limitation as “the surface tension relationship.” For brevity, we will do the same.

IPR2015-01157

Patent 8,889,083 B2

surfactant to achieve controlled or optimized plug flow.”). Notably, Dr. Huck did not testify that concentrations between 0.2 and 5%, as disclosed by Quake, would achieve the claimed surface tension relationship. Ex. 2027, 256:2–257:4.

This, we conclude, is a change in Dr. Huck’s rationale for unpatentability: from an actual disclosure in Quake of the claimed surface tension relationship, to a modification of Quake through routine optimization to achieve the surface tension relationship. As such, it is improper to raise this for the first time in a reply declaration. *See Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, Case IPR2013-00517, 2015 WL 996355, at *9 (PTAB Feb. 11, 2015) (excluding reply that “chang[ed] the unpatentability rationale from express reliance on . . . conditions, to asserting that those conditions would have been obvious to modify”). To permit 10X to present this changed theory for the first time in Dr. Huck’s reply testimony would deprive Chicago of a meaningful opportunity to respond. *See Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1080 (Fed. Cir. 2015) (“A patent owner . . . is undoubtedly entitled to notice of and a fair opportunity to meet the grounds of rejection.”). We will not consider 10X’s routine optimization argument⁷ in evaluating the proposed ground of unpatentability.

⁷ We note that even if we were to consider it, routine optimization is of dubious applicability in this case. At oral hearing, counsel argued that Example 12 of Quake tests “several different concentrations of surfactant and finds that 2 percent is best.” Tr. 74:21–24, 76:5–10. This is not an accurate description of Example 12. Quake does not evaluate the effect of using different surfactant concentrations—it merely states that different

IPR2015-01157

Patent 8,889,083 B2

e. Conclusion

As we noted above, if the Board determines that a reply brief or testimony exceeds the proper scope of reply, we typically will not parse the paper in an attempt to separate proper from improper portions. Trial Practice Guide, 77 Fed. Reg. at 48,767. In this case, however, no such parsing is required because Chicago's paper identifying the allegedly improper testimony sets forth the particular paragraphs it contends are objectionable. For the reasons given, we agree that ¶¶ 45–48, 71–74, and 76–78 of Dr. Huck's Second Declaration exceed the proper scope of reply testimony, and will not consider those portions in reaching our decision in this trial.

2. New Argument in Reply

At oral hearing, counsel for Chicago contended that—in addition to the portions of Dr. Huck's Second Declaration discussed above—the Reply submitted by 10X contained new arguments and issues and therefore was an improper reply. Tr. 40–48. Before addressing these contentions, however, we summarize the relevant arguments and evidence as presented by 10X in its briefs, and in Dr. Huck's testimony.

concentrations of 0.5, 1.0, or 2.0% were tried—and it does not characterize any concentration as “best.” Ex. 1004 ¶ 300. Furthermore, even if Quake can be said to evaluate surfactant concentrations, there is no disclosure in the reference indicating that changing concentration resulted in a change in the claimed surface tension relationship. *See In re Antonie*, 559 F.2d 618, 620 (CCPA 1977) (variable must be recognized as result-effective before routine optimization applies).

IPR2015-01157

Patent 8,889,083 B2

a. 10X's Arguments Regarding Surface Tension Relationship

Central to the dispute between the parties are two limitations of the independent claims: first, “a fluorinated surfactant comprising a hydrophilic head group”; and second, that the surfactant is “present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.” In its Petition, 10X argued that Quake discloses the first limitation in paragraphs 116 and 117, and in particular quoted paragraph 117: “**The fluids used in the invention may contain additives**, such as agents which reduce surface tensions (**surfactants**). **Exemplary surfactants include . . . fluorinated oils, . . .**” (emphasis and ellipses in original). Pet. 19; *see also id.* at 22–23. Dr. Huck’s Declaration supporting the Petition cited and quoted the same portion of Quake. Ex. 1002, claim chart following ¶ 53 (pg. 33).

For the surface tension relationship limitation, however, 10X relied on a different portion of Quake—namely, paragraphs 94 and 118, which discuss coating the channel walls with surfactant. From paragraph 94, 10X quoted Quake’s statement that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels (and coverslip, if used) may have a coating which minimizes adhesion. . . . Alternatively, the channels may be coated with a surfactant.” Pet. 20–21 (emphasis omitted). From paragraph 118, 10X includes the following quote: “The **channels may also be coated** with additives or agents, such as surfactants, TEFLON, or **fluorinated oils** such as octadecafluorooctane . . . or fluorononane.” *Id.* (emphasis and ellipses in original). Again, Dr. Huck similarly relies on paragraphs 94 and 118 of

IPR2015-01157

Patent 8,889,083 B2

Quake to disclose the surface tension relationship. Ex. 1002, claim chart following ¶ 53 (pg. 35); *see id.* ¶ 61.

During cross-examination at his first deposition, Dr. Huck stated that paragraph 117 is the only portion of Quake that discloses “how a surfactant can be used to assist flow of a droplet.” Ex. 2026, 150:23–151:6. Dr. Huck also agreed that he was not relying on the wall-coating surfactants disclosed in paragraphs 94 or 118 to support his obviousness theory. *Id.* at 156:8–13, 157:1–8. Rather, Dr. Huck repeatedly cited paragraph 117’s disclosure that surfactants “aid in controlling or optimizing droplet size, flow, and uniformity” as evidence of the claimed surface tension relationship. *See, e.g., id.* at 99:25–100:4; 112:8–12; 133:16–20.

Similarly, in its Reply, 10X no longer cites the wall-coating surfactants of paragraphs 94 or 118 as disclosing the claimed surface tension relationship. Nor does 10X cite to Dr. Huck’s first Declaration regarding these paragraphs. Pet. Reply 10–11. 10X instead relies on Dr. Huck’s deposition testimony regarding paragraph 117’s “controlling or optimizing droplet size, flow, and uniformity” statement. *Id.* (citing Ex. 2026). Nor does Dr. Huck’s Second Declaration rely on paragraphs 94 and 118, instead citing to paragraph 117 as evidence of the surface tension relationship. Ex. 1087 ¶¶ 72–74.

b. Analysis

An *inter partes* review trial before the Board is a formal adjudication under the Administrative Procedure Act; as such, the parties to the trial are guaranteed certain procedural protections. *See Belden*, 805 F.3d at 1080. Significant among these is that parties must be given notice of the “matters

IPR2015-01157

Patent 8,889,083 B2

of fact and law asserted,” and the opportunity to meaningfully respond. *Id.* For this reason, the Federal Circuit has held that we may not base our decision on patentability on late-arising factual assertions or theories. *See Dell Inc. v. Accelaron, LLC*, 818 F.3d 1293, 1301 (Fed. Cir. 2016). In *Dell*, “an opportunity to respond was needed when the petitioner, to make its anticipation showing, newly pointed to a previously unmentioned portion of the allegedly anticipatory prior-art patent, even though it had earlier focused extensively on other portions of that prior-art patent.” *In re Nuvasive Inc.*, No. 2015-1672, 2016 WL 6608999, at *5 (Fed. Cir. Nov. 9, 2016) (discussing *Dell*, 818 F.3d at 1301).

To be sure, we are not “limited to citing only portions of the prior art specifically drawn to [our] attention” in a petition. *Id.* at *4. But where the newly cited portions are “sufficiently distinct” from those previously presented by a party, the opposing party is entitled to the opportunity to respond. *Id.* at *5. Therefore, while we must consider the disclosure of a prior art reference as a whole, this does not mean that we may permit 10X to change its theory of unpatentability during trial.

10X argues that it has not changed its position, and has consistently cited paragraph 117 of Quake from the outset. Tr. 71:13–24; Pet. Reply 23. According to 10X’s counsel, the Petition cited paragraph 117 “in general, and [in] its entirety, repeatedly.” Tr. 73:8–9. There is no dispute that the Petition cited paragraph 117 of Quake. But the proposition for which paragraph 117 was cited in the Petition was the disclosure of fluorinated surfactants in the carrier fluid. Pet. 19–20. The paragraph’s statement that surfactants “aid in controlling or optimizing droplet size, flow, and

IPR2015-01157

Patent 8,889,083 B2

uniformity” is quoted nowhere in the Petition; nor is paragraph 117 relied upon in the sections of the Petition pertaining to the surface tension relationship, other than to establish the presence of the surfactant itself.

The change in 10X’s position is not insignificant, or a mere change in emphasis from one portion of Quake to another. Rather, it signals a change in the theory of unpatentability advanced in the Petition upon which we instituted trial. In its Petition, 10X argued that the claimed surface tension relationship is met when droplets do not adhere to the walls of the channel, and cites Quake’s disclosure (in paragraph 118) of surfactants *coating the walls* as evidence that the surfactant is causing the surface tension relationship. This is the theory of unpatentability upon which we instituted trial:

Petitioner does not contend that Quake specifically discloses the relationship between surface tension at the plug-fluid/microchannel interface and the plug-fluid/carrier fluid interface. Rather, Dr. Huck notes Quake’s disclosure that a surfactant may be used to cause the surfactant to *coat the channel walls*. . . . According to Dr. Huck, a person of ordinary skill in the art would have understood this to mean that the surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.

Dec. on Inst. 16–17 (emphasis added).

Starting with Dr. Huck’s deposition and continuing through the Reply, however, 10X’s theory is that the surface tension relationship is proven by the presence of “free-flowing droplets,” and that Quake discloses in paragraph 117 that surfactants *in the carrier fluid* “aid in controlling or optimizing droplet size, flow and uniformity.” As counsel for 10X conceded at oral hearing, paragraphs 117 and 118 “disclose two *separate* uses of

IPR2015-01157

Patent 8,889,083 B2

surfactants.” Tr. 21:4–9 (emphasis added). It would be unfair to Chicago if we were to institute trial on one of these uses of surfactants, and then base our finding of unpatentability on the other, separate use.

We consider it of no moment that the change in theory was first signaled during Dr. Huck’s deposition, prior to Chicago filing its Patent Owner Response. Nor do we find it significant that, in its Patent Owner Response, Chicago included a section preemptively addressing 10X’s potential change in theory. PO Resp. 42–46. Until 10X filed its Reply, Chicago did not know whether 10X would adopt Dr. Huck’s testimony on paragraph 117 and his abandonment of paragraphs 94 and 118. Furthermore, the Board does not consider arguments made only in witness testimony and not explained sufficiently in a paper. *Cf. Cisco Sys., Inc. v. C-Cation Techs., LLC*, Case IPR2014-00454, slip op. at 10 (PTAB Aug. 29, 2014) (Paper 12) (informative) (declining to consider arguments not made in a petition and only incorporated by reference from expert declaration). Chicago was not fully on notice of 10X’s new theory of unpatentability until the Reply, when it adopted Dr. Huck’s cross-examination testimony.

c. Conclusion

We have determined that the Reply and Dr. Huck’s supporting Second Declaration raise new issues and arguments for the first time. The citation to the new sentence of paragraph 117 of Quake is a change in 10X’s theory of unpatentability. Furthermore, as discussed above, both the Declaration and the Reply rely on a theory of routine optimization that is not set forth in the Petition. *See* Pet. Reply 11. The Reply and Second Declaration, therefore,

IPR2015-01157

Patent 8,889,083 B2

exceed the proper scope of reply permitted under our Rules. *See* 37 C.F.R. § 42.23(b).

We determined above that we would not rely on particular sections of Dr. Huck’s Second Declaration objected to by Chicago. For similar reasons, we will not rely on the Reply to the extent it raises the new arguments discussed above. Though we would be permitted, under our Rules and precedent, to exclude the entirety of the Reply, on the facts of this case we consider it sufficient to not consider the the newly-raised theories of unpatentability regarding paragraph 117 of Quake and routine optimization raised in Sections II, III, and VIII of the Reply. *See Ariosa Diagnostics v. Verinata Health, Inc.*, 805 F.3d 1359, 1367 (Fed. Cir. 2015) (affirming Board’s exclusion of certain arguments raised for the first time in Reply). In evaluating 10X’s asserted ground of unpatentability over Quake and Ramsey, we will rely on the theory of unpatentability as set forth in the Petition and Dr. Huck’s first Declaration, on which we instituted trial.

3. Fluorinated Surfactant Having a Hydrophilic Head Group

Turning to the merits of the ground of unpatentability, we first focus on the limitation—present in all independent claims—that requires that the carrier fluid comprise “a fluorinated surfactant comprising a hydrophilic head group.” 10X asserts that Quake discloses this element. Pet. 19–20 (citing Ex. 1004 ¶¶ 116–117). Quake discloses in those paragraphs that its carrier fluid “may contain additives, such as agents which reduce surface tensions (surfactants)” and that “[e]xemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.” Ex. 1004 ¶ 117.

IPR2015-01157

Patent 8,889,083 B2

Dr. Huck testifies that “[t]he term surfactant is a contraction of the descriptive phrase ‘surface active agent.’ . . . because they concentrate at interfacial regions such as oil-water or liquid-solid interfaces.” Ex. 1002 ¶ 42. In aqueous systems such as those disclosed in the ’083 patent and Quake, Dr. Huck testifies that the surfactant is understood to reside at the oil-water boundary because it has a hydrophilic head group and a hydrophobic tail group. *Id.* (citing Ex. 1022, 3). Dr. Olmsted, who has significant experience in interfacial phenomena including surfactants, agrees, and further notes that Tween and Span—mentioned in the same sentence of Quake cited by 10X—have hydrophilic head groups. Ex. 1085 ¶¶ 26–29.

In response, Chicago notes that Dr. Huck admits surfactants, by definition, do not require a hydrophilic head group. PO Resp. 27. Furthermore, Chicago argues that Quake’s disclosure of surfactants are to coat the channel walls, meaning that they reside at the channel wall/carrier fluid interface. *Id.* at 28–29. In such situations—where the interface is between two hydrophobic materials—Dr. Huck agreed that he would not use a surfactant with a hydrophilic head group. *Id.* (citing Ex. 2026, 102:17–23). Dr. Sia’s testimony supports these arguments. Ex. 2028 ¶¶ 71–87.

We agree with Chicago that Quake does not explicitly disclose that the surfactants of paragraph 117 have a hydrophilic head group, and that “surfactants” in general include surface active agents both with and without a hydrophilic head group. Nevertheless, we find that a person of skill in the art would understand the surfactants disclosed in paragraph 117 may include a hydrophilic head group. Dr. Olmsted notes, and Chicago does not dispute,

IPR2015-01157

Patent 8,889,083 B2

that the other surfactants listed as “exemplary”—namely, Tween and Span—have hydrophilic head groups. Ex. 1085 ¶ 27. Furthermore, the sentence characterizes the surfactants as “soluble in oil relative to water,” from which Dr. Olmsted concludes that the oil-water boundary is the surface where the surfactants are expected to reside. *Id.* ¶ 29. While Chicago is correct that other portions of Quake—for example, as discussed extensively above, paragraph 118—disclose surfactants that coat the channel walls, we do not understand paragraph 117’s surfactants to be so limited.⁸

For this reason, we find credible Dr. Huck’s and Dr. Olmsted’s testimony that a person of ordinary skill in the art would understand paragraph 117’s disclosure to refer to surfactants having a hydrophilic head group. We find that Quake discloses fluorinated surfactants that reside at the droplet-carrier fluid interface, and which have a hydrophilic head group.

4. Surface Tension Relationship

Having found the claimed surfactant disclosed in Quake, we now address the challenged independent claims’ requirement that the surfactant is “present in a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-

⁸ In any event, even if Quake’s surfactants were limited to those coating the channel walls, Quake discloses that some channels may be made of hydrophilic materials such as urethane. Ex. 1004 ¶ 118 (“Channels of the invention may be formed from . . . urethane compositions” and “hydrophilic properties of urethane.”); *see also id.* ¶ 216 (“PDMS device is placed in a hot bath of HCl to make the surface hydrophilic”). In such applications, the surfactant coating these hydrophilic channel walls would reasonably be expected to have a hydrophilic head group.

IPR2015-01157

Patent 8,889,083 B2

fluid/carrier fluid interface.” 10X again relies on Quake for this disclosure, in particular paragraphs 94 and 118 of the reference.

10X concedes that Quake does not explicitly disclose the surface tension relationship. Tr. 8:13–20. Rather, 10X infers that the surface tension relationship is met because of the presence of droplets that do not adhere to the channel walls. *Id.* at 10:9–13; Pet. 24. 10X then ties this non-adhesion of droplets to the presence of surfactants coating the channel walls, as disclosed in paragraphs 94 and 118 of Quake. Pet. 25 (“The combination of Quake and Ramsey teaches how to avoid adhesion of plugs to the wall. Quake discloses use of fluorinated oils such as surfactants in carrier fluid and discloses use of such surfactants for coating the walls of microchannels.”). As discussed at length above, Dr. Huck testified in his first Declaration in support of this inference of the surface tension relationship. Ex. 1002 ¶¶ 59–61.

The theory advanced by 10X—that the surface tension relationship can be inferred from the lack of adhesion of droplets—suffers from at least two failings. First, as discussed above, to establish the presence of fluorinated surfactants with a hydrophilic head group, 10X relies on paragraph 117 of Quake, which it argues discloses surfactants residing at the oil-water interface between the droplets and the carrier fluid. Indeed, this reliance is crucial to its inference that the surfactants have a hydrophilic head group. But when it comes to inferring that the claimed surface tension relationship is present, 10X points to a different disclosure of surfactants coating the channel walls. As counsel conceded, the disclosures of paragraphs 117 and 118 “disclose two separate uses of surfactants.” Tr.

IPR2015-01157

Patent 8,889,083 B2

21:8–9. 10X cannot have it both ways, arguing that the hydrophilic head group is implied because the surfactant is at the oil-water interface, while also arguing that the surface tension relationship is implied because the surfactant coats the channel walls.

Furthermore, even if we were to accept 10X’s reliance on the surfactant coating the channel walls, its proposed inference is not supported by the record or logic. While it may be the case that if the surface tension relationship is present, droplets do not adhere to the wall, to infer the reverse would be affirming the consequent.⁹ In other words, we cannot infer from the fact that droplets do not adhere to the channel wall that the claimed surface tension relationship is necessarily present, because there are other possible causes of non-adherence. As Chicago correctly notes, Quake discloses multiple reasons why a droplet may not adhere to a channel wall, including droplet shape (“about four times as long as [it is] wide”) and size (“[d]roplet adherence is overcome when the droplet is massive enough in relation to the channel size”). PO Resp. 24–25 (citing Ex. 1004 ¶ 92).

10X attempts to distinguish these causes of non-adherence in Quake’s paragraph 92 by arguing that they pertain only to “a situation where a droplet has actually touched the channel wall and then breaks off. That is not a free-flowing droplet. A free-flowing droplet is one that doesn’t adhere to the wall and flows through the channel.” Tr. 11:15–12:5. The record does not support such a distinction. Indeed, paragraph 92 uses the exact

⁹ The “fallacy of affirming the consequent” is the following logical fallacy: “If cause, then effect.” “Effect, therefore cause.” The fallacy occurs because there may be other causes that lead to the same effect.

IPR2015-01157

Patent 8,889,083 B2

term “free-flowing droplet,” discussing a “typical free-flowing droplet” of a certain shape, such that it “overcome[s] channel adherence and move[s] freely through the microfluidic device.” Ex. 1004 ¶ 92. In sum, we cannot infer from the evidence adduced by 10X—that Quake discloses droplets that do not adhere to channel walls—that the claimed surface tension relationship is present.¹⁰

For these reasons, we cannot conclude from the arguments advanced in the Petition that the claimed surface tension relationship is disclosed by Quake.

5. Conclusion

10X has not proven by a preponderance of the evidence that Quake teaches the surface tension relationship limitation of the challenged claims. Nor does 10X allege that Ramsey teaches or suggests this limitation. For these reasons, we cannot conclude that 10X has carried its burden of proving that claims 1–31 are unpatentable over the combined disclosures of Quake and Ramsey.

¹⁰ We note that we would reach the same conclusion even if we had not excluded portions of 10X’s Reply. 10X’s new theory of unpatentability—which relies on paragraph 117 as evidence of the surface tension relationship—suffers from the same infirmity as its original theory; namely that we cannot infer from the fact that “[s]urfactants may aid in controlling or optimizing droplet size, flow and uniformity” (Ex. 1004 ¶ 117) that the claimed surface tension relationship is present.

IPR2015-01157

Patent 8,889,083 B2

C. Obviousness over Quake, Ramsey, and Green

Claims 18 and 24 further require particular fluorinated oils as the carrier fluid. Petitioner argues that these claims are unpatentable as having been obvious over the combined disclosures of Quake, Ramsey, and Green. Pet. 50–52. Petitioner contends that, given the disclosure in Ramsey of perfluorocarbon segmenting fluids, a person of ordinary skill in the art would have consulted Green, which lists perfluorocarbons used in various applications, including the perfluorocarbons recited in claims 18 and 24. *Id.* at 51.

10X does not contend, nor do we find, that Green remedies the deficiency in Quake noted above. In other words, the record does not establish that Green teaches or suggests the claimed surface tension relationship. For this reason, 10X has not met its burden of proving that claims 18 and 24 are unpatentable over the combined disclosures of Quake, Ramsey, and Green.

D. Motion to Exclude

Chicago moves to exclude various exhibits filed by 10X, namely Exhibits 1037, 1043, 1045, 1047, 1050, 1051–1056, 1072, 1081, 1088–1092, and portions of Dr. Huck’s testimony (Exs. 1002 and 1087). Mot. 1. The basis for the Motion is Chicago’s prior objections to these exhibits on grounds of hearsay (FRE 802), prejudice (FRE 403), or unreliability (FRE 702). Even having considered these objected-to exhibits, however, we have found in Chicago’s favor on the merits of 10X’s patentability challenge. As such, the grant or denial of Chicago’s Motion would have no effect on the

IPR2015-01157

Patent 8,889,083 B2

outcome of the proceeding. We, therefore, dismiss the Motion to Exclude as moot.

III. CONCLUSION

For the foregoing reasons, based on a review of the complete record developed during trial, we conclude that 10X has not proven, by a preponderance of the evidence, that claims 1–31 are unpatentable.

IV. ORDER

Accordingly, it is:

ORDERED that claims 1–31 of U.S. Patent No. 8,889,083 B2 *have not been proven unpatentable*;

FURTHER ORDERED that, pursuant to 35 U.S.C. § 318(b), upon expiration of the time for appeal of this decision, or the termination of any such appeal, a certificate shall issue confirming the patentability of claims 1–31 of U.S. Patent No. 8,889,083 B2;

FURTHER ORDERED that Patent Owner's Motion to Exclude (Paper 36) is *dismissed as moot*; and

FURTHER ORDERED that, because this is a Final Written Decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

IPR2015-01157

Patent 8,889,083 B2

For PETITIONER:

Eldora Ellison

eellison-PTAB@skgf.com

Deborah Sterling

dsterlin-PTAB@skgf.com

For PATENT OWNER:

Derek Walter

derek.walter@weil.com

Elizabeth Weiswasser

elizabeth.weiswasser@weil.com

Adrian Percer

adrian.percer@weil.com

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

10X GENOMICS, INC.

Petitioner

v.

THE UNIVERSITY OF CHICAGO

Patent Owner

U.S. Patent No. 8,889,083 to Ismagilov *et al.*

Issue Date: November 18, 2014

Title: Device and Method for Pressure-Driven
Plug Transport and Reaction

Inter Partes Review No. Unassigned

**Petition For *Inter Partes* Review of U.S. Patent No. 8,889,083 Under 35 U.S.C.
§§ 311-319 and 37 C.F.R. §§ 42.1-.80, 42.100-.123**

Mail Stop "PATENT BOARD"

Patent Trial and Appeal Board

U.S. Patent and Trademark Office

P.O. Box 1450

Alexandria, VA 22313-1450

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

TABLE OF CONTENTS

I.	Introduction.....	1
II.	Grounds for standing (37 C.F.R. § 42.104(a))	2
III.	Statement of the precise relief requested and the reasons therefor	2
IV.	Overview.....	3
	A. Person of ordinary skill in the art ("POSA") and state of the art	3
	1. Analyzing biological samples and performing chemical reactions in microfluidic devices was well-known in the art by May 2002.....	4
	B. The '083 patent	8
V.	Claim construction.....	9
VI.	Identification of challenge (37 C.F.R. § 42.104(b)).....	17
	A. Ground 1: Claims 1-31 would have been obvious over Quake and Ramsey	18
	B. Ground 2: Claims 18 and 24 would have been obvious over Quake, Ramsey and Green	50
	C. Objective indicia do not support patentability	53
	1. No acquiescence by licensing	54
	2. No commercial success	55
	3. No long-felt but unmet need or failure of others.....	56
	4. No industry praise	57
	5. No unexpectedly superior results	58
VII.	Conclusion	59
VIII.	Mandatory notices (37 C.F.R. § 42.8(a)(1)).....	59

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

I. Introduction

U.S. Patent No. 8,889,083 ("the '083 patent") claims microfluidics systems and methods that were known in the art. The claims recite non-fluorinated microchannels and a carrier fluid containing a fluorinated oil with a fluorinated surfactant. Indeed, the Examiner concluded that a fluorinated surfactant in the microfluidic device provided a reason to allow the '083 patent claims. But the claimed components—including fluorinated surfactants—had been used in microfluidics systems before 2002, the earliest possible priority date of the '083 patent¹. The '083 patent claims also recite that the surface tension between a plug (an aqueous volume) being carried in the system and the wall of the system is higher than the surface tension between the plug and the carrier fluid. The '083 patent alleges that this difference in surface area is achieved by adding a fluorinated surfactant to the carrier fluid. The '083 patent also discusses how this difference in surface tension prevents plugs from adhering to the wall of the system. But this too is nothing new; using fluorinated surfactants to prevent plugs from adhering to channels in microfluidics systems was known prior to 2002, and prior art systems applied this known solution.

¹ Petitioner does not concede that the '083 patent is entitled to a May 9, 2002 filing date; however, the '083 patent cannot be entitled to any earlier filing date.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

The prior art references Quake and Ramsey cited herein show that the components of the microfluidics systems and methods claimed in the '083 patent were known to a person of ordinary skill in the art ("POSA"). Quake shows that microfluidics systems comprising a non-fluorinated microchannel, a carrier fluid fluorinated oil surfactant and at least one plug were known in the art. And Ramsey shows that fluorinated oils are preferable carrier oils for use with biological molecules in microfluidics systems. A POSA would have had a reason to use the fluorinated carrier oil of Ramsey in the systems of Quake, as Quake's system is for analysis of biological molecules and as Quake already teaches the use of fluorinated oil surfactants. And a POSA would know how to achieve the claimed differences in surface tension that allegedly add novelty to the claims, because these surface tension differences only represent surface tensions under which the aqueous plugs do not adhere to the wall of the microchannel. *Inter partes* review of all claims of the '083 patent should be instituted on the grounds below.

II. Grounds for standing (37 C.F.R. § 42.104(a))

Petitioner certifies that the '083 patent is available for IPR and Petitioner is not barred or estopped from requesting IPR of any of the challenged claims.

III. Statement of the precise relief requested and the reasons therefor

The Office should institute IPR under 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42.1-.80 and 42.100-42.123, and cancel claims 1-31—all claims—of the '083 patent as unpatentable under 35 U.S.C. § 103. Petitioner's full statement of the

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

reasons for the relief requested is set forth in detail in § VI.

IV. Overview

A. Person of ordinary skill in the art ("POSA") and state of the art

A POSA is a hypothetical person who is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art, and is a person of ordinary creativity. A POSA in the field of microfluidic devices and the methods of using such devices would have had knowledge of the scientific literature concerning microfluidic devices and the methods of using such devices before May 9, 2002. A POSA would have had knowledge of strategies for performing chemical and biological analysis in microfluidic devices. Typically, a POSA would have had a Ph.D. in chemistry, biochemistry, mechanical engineering, or a related discipline, with two years of experience in using, designing or creating microfluidic devices. Alternately, a POSA could have had a M.S. or bachelor's degree in one of these disciplines with four or five years of additional relevant experience, respectively. A POSA would have known how to research the scientific literature in fields relating to microfluidics, including fluid dynamics, microscale reactions, chemistry, biochemistry, and mechanical engineering. Also, a POSA may have worked as part of a multidisciplinary team and drawn upon not only his or her own skills, but also taken advantage of certain specialized skills of others in the team, e.g., to solve a given problem. For example, a chemist, engineer, and a biologist may have been part of a team. (GEN1002, ¶13.)

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

By May 9, 2002, the state of the art included the teachings provided by the references discussed in this petition. Additionally, a POSA would have been aware of other important references relating to performing reactions, including PCR, in microfluidic devices.

1. Analyzing biological samples and performing chemical reactions in microfluidic devices was well-known in the art by May 2002

Since at least 1957, capillary systems for analyzing biological samples were well-known in the art. (GEN1008, 1:15-19, 2:62-70, Fig. 3²; GEN1002, ¶15.) In these systems, a biological sample was introduced into a main capillary channel. (GEN1008, 4:6-18; GEN1002, ¶15.) Pockets of air were injected into the channel in an alternating fashion with each sample. (GEN1008, 4:29-34; GEN1002, ¶15.) This injection method resulted in a segmented stream composed of alternating segments of air and biological sample, *i.e.*, slugs of air and biological sample. (GEN1008, 4:37-42; GEN1002, ¶15.) Similar systems were also developed that

² Citations to patent literature provided as GEN10XX, YYY:Z-Z indicate citations to column Y, at lines Z-Z. For example, GEN10XX, 1:1-10 indicates column 1, lines 1-10. Citations to non-patent literature provided as GEN10XX, Y:Z indicate citations to page number Y, at column number Z. For example, GEN10XX, 10:1 indicates p. 10, at column 1.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

injected an immiscible fluid, such as mineral oil, instead of air to create the segmented stream—resulting in alternating slugs of immiscible fluid and biological sample. (GEN1009, 3:11-37; GEN1002, ¶15.)

However, these systems had drawbacks. For instance, it was well-known that the contents of slugs containing biological samples or chemical reactants could adhere to channel walls and contaminate the next successive slug. (GEN1009, 3:11-37; GEN1008, 4:42-54; GEN1010, 1:57-59; GEN1002, ¶16.) These systems could minimize cross-contamination, but did not completely cure the problem. (GEN1010, 1:68-2:4; GEN1002, ¶16.)

To solve this problem of cross-contamination, Smythe *et al.* developed a fluid analysis system, in which a sample fluid was aspirated into a channel made of fluorinated hydrocarbon. (GEN1010, 1:68-2:4, 2:35-61; GEN1002, ¶17.) In Smythe's system, a silicone fluid flowing within the fluorinated hydrocarbon channel wets the channel walls and forms a film on the walls. (GEN1010, 2:35-61 and Fig. 1; GEN1002, ¶17.) This method prevented the loss of material from the volumes of sample fluid, and thus cross-contamination. (GEN1010, 2:50-58; GEN1002, ¶17.) Smythe's method resulted in a volume of sample fluid encapsulated by a thin film of silicone fluid, which would travel within the film of silicone fluid and not touch or adhere to the inner walls of the fluorinated hydrocarbon channel. (GEN1010, 2:35-61, Fig. 1; GEN1002, ¶17.) However, this

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

system also required injecting encapsulated volumes of air or an inert gas between the encapsulated volumes of sample fluid to prevent coalescence of the encapsulated volumes of sample fluid. (GEN1010, 2:62-71; GEN1002, ¶17.)

In 1984, Shaw Stewart developed a microfluidic system for performing chemical reactions. (GEN1011, Abstract; GEN1002, ¶18.) Shaw Stewart explicitly stated that an object of his system was "to reduce or eliminate contamination of the reactants[.]" (GEN1011, p. 4.) To achieve this goal, Shaw Stewart's system injected an aqueous fluid containing a chemical reactant through one inlet into a main channel in which a carrier fluid, such as a fluorinated oil, was flowing. (GEN1011, Abstract, pp. 5-6; GEN1002, ¶18.) As a result of this process, volumes of the aqueous fluid were sheared off into droplets that were encapsulated by the carrier fluid present in the main channel. (GEN1011, pp. 5-6; GEN1002, ¶18.) This process was repeated to inject a second chemical reactant through a second channel. (GEN1011, pp. 5-6; GEN1002, ¶18.) The droplets containing the first reactant and the second reactant were then coalesced to combine the reactants. (GEN1011, pp. 5-6; GEN1002, ¶18.) Shaw Stewart additionally teaches that any remaining "[c]ontamination of the walls can be reduced by: ... constructing conduits with walls coated with or constructed from a material which repels the reactant phase but attracts the carrier phase[.]" (GEN1011, p. 9.) Thus, a POSA knew in 1984 that preventing adhesion of plugs to a microfluidic channel was

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

important for preventing contamination between plugs. (GEN1002, ¶19.)

Building upon the microfluidic systems described above, Quake *et al.* developed a system similar to that of Shaw Stewart in 2000. (GEN1004; GEN1005; GEN1002, ¶20.) Quake's system, like Shaw Stewart's, sheared off an aqueous fluid—flowing through an inlet and into a main channel—into droplets encapsulated by an immiscible fluid. (GEN1004, ¶¶[0015], [0100]; *see* GEN1005, 6:19-29, 30:2-3; GEN1002, ¶20.) Quake's droplets can also conform to the size and shape of the channel, while maintaining their respective volumes. (GEN1004, ¶¶[0091]-[0092]; *see* GEN1005, 28:4-6; GEN1002, ¶20.) And Quake's system allowed for the continuous production of encapsulated droplets at a fixed frequency. (GEN1004, ¶[0290]; GEN1002, ¶20.)

Quake teaches the use of surfactants to prevent adhesion of droplets to the device wall and thus prevent cross-contamination. Quake teaches that "[t]o prevent material ... from adhering to the sides of the channels, the channels ... may **have a coating which minimizes adhesion**. Such a coating may be intrinsic to the material from which the device is manufactured, Alternatively, the **channels may be coated with a surfactant**." (GEN1004, ¶[0094], emphasis added; *see* GEN1005, 28:21-25.) Thus, Quake's system functions to prevent cross-contamination. (GEN1004, ¶[0006]; *see* GEN1005, 3:23-4:3; GEN1002, ¶21.) And Quake also envisioned that its system would be adaptable to analysis of

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

biomolecules, such as by PCR. (GEN1004, ¶[0080]; *see* GEN1005, 24:12-16; GEN1002, ¶27.) Thus, by 2002, Quake provided a system that addressed issues of cross-contamination and allowed for analysis of biomolecules. (GEN1002, ¶27.)

B. The '083 patent

Against this background, the '083 patent was filed on October 30, 2006. (GEN1001.) The '083 patent asserts its earliest priority claim to May 9, 2002, and is assigned to the University of Chicago ("Patent Owner"), according to the Office's electronic-assignment records. The '083 patent claims microfluidic systems and methods of conducting reactions in microfluidic systems where the systems comprise a non-fluorinated microchannel, a carrier fluid comprising a fluorinated oil and a fluorinated surfactant, at least one plug in the microchannel encased by the carrier fluid, where the surface tension at the plug-fluid/microchannel wall interface is higher than the surface tension at the plug-fluid/carrier fluid interface. (GEN1001, 73:10-75:4.)

Independent claims 1, 20 and 31 also require that "fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface." (GEN1001, 73:18-21.) As the surface tension is higher at the plug-fluid/wall interface than the plug-fluid/carrier fluid interface, the plug-fluid will be repelled by the wall and will not wet, or adhere to, the wall. (GEN1002, ¶31.)

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 20 is a method of conducting an autocatalytic reaction in such plugs.

V. Claim construction

Unless otherwise construed herein, the terms of claims 1-31 must be given their broadest reasonable interpretation ("BRI"), as understood by one of ordinary skill in the art in view of the '083 patent's specification. *See* 37 C.F.R. § 42.100(b).

Plugs of aqueous fluid: The '083 patent defines plugs as follows:

"Plugs" in accordance with the present invention are formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible. The flow of the fluids in the device is induced by a driving force or stimulus that arises, directly or indirectly, from the presence or application of, for example, pressure, radiation, heat, vibration, sound waves, an electric field, or a magnetic field. Plugs in accordance with the present invention may vary in size but when formed, their cross-section should be substantially similar to the cross-section of the channels in which they are formed. When plugs merge or get trapped inside plug traps, the cross-section of the plugs may change. For example, when a plug enters a wider channel, its cross-section typically increases. (GEN1001, 9:6-19.) The specification further defines the term "plug-fluid" as "a fluid wherein or using which a reaction or precipitation can occur," and the term "carrier-fluid" as "a fluid that is immiscible with a plug-fluid." (GEN1001, 9:47-49 and 7:21-23.) Additionally, the specification further describes

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

the relationship between the carrier-fluid and plug fluid, stating:

The carrier-fluid preferably wets the walls of the channels preferentially over the plugs. If this condition is satisfied, the plug typically does not come in contact with the walls of the channels, and instead remains separated from the walls by a thin layer of the carrier-fluid. Under this condition, the plugs remain stable and do not leave behind any residue as they are transported through the channels.

(GEN1001, 20:41-47; GEN1002, ¶34.)

The '083 patent states that the plugs "may be in the form of plugs comprising an aqueous plug-fluid containing one or more reagents and/or one or more products formed from a reaction of the reagents, wherein the aqueous plug-fluid is surrounded by a non-polar or hydrophobic fluid such as an oil." (GEN1001, 9:24-29.) As such, a POSA would have understood the BRI of "plugs of the aqueous fluid" to be: "Volumes of an aqueous fluid surrounded by a carrier fluid in which it is substantially immiscible, such as a non-polar or hydrophobic fluid, e.g., an oil. The diameter of a plug is substantially similar to the cross-section of the channel in which it is formed and the aqueous fluid is separated from the channel wall by a thin film of carrier fluid." (GEN1002, ¶36.)

Non-fluorinated microchannel: The '083 patent defines "channel" as "a conduit that is typically enclosed, although it may be at least partially open, and that allows the passage through it of one or more types of substances or mixtures...A channel is typically made of a suitable material such as a polymer,

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

metal, glass, composite, or other relatively inert materials." (GEN1001, 7:24-36.)

The '083 patent further states that "[a]s used herein, the term 'channel' includes **microchannels** that are of dimensions suitable for use in devices." (GEN1001, 7:36-38, emphasis added.) When discussing the dimensions of microchannels, the '083 patent does so in the context of the size of plugs that are formed by conforming to the microchannel dimension. For example, the '083 patent states:

The length of the plugs can be controlled such that their sizes can range from, for example, about 1 to 4 times a cross-sectional dimension (d , where d is a channel cross-sectional dimension) of a channel using techniques such as varying the ratio of the plug-fluids and carrier-fluids or varying the relative volumetric flow rates of the plug-fluid and carrier-fluid streams. ... In one approximation, the volume of a plug is taken equal to about $2xd^3$, where d is a cross-sectional dimension of a channel. Thus, the plugs can be formed in channels having cross-sectional areas of, for example, from 20×20 to $200 \times 200 \mu\text{m}^2$, which correspond to plug volumes of between about 16 picoliters (pL) to 16 nanoliters (nL). The **size of channels** may be increased to **about 500 μm** (corresponding to a volume of about 250 nL) or more. The channel size can be reduced to, for example, **about 1 μm** (corresponding to a volume of about 1 femtoliter).

(GEN1001, 19:16-35 (emphasis added).) Thus a POSA would understand that the claimed microchannels are from about 1-500 μm in size. (GEN1002, ¶40.)

The term "non-fluorinated" was added during prosecution of the '083 patent to qualify the term "microchannel" in an amendment intended to overcome an

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

obviousness rejection. (GEN1023, 1554-1559.) In discussing the amended claims, Applicant pointed to the disclosure at page 30, lines 18-21 of the specification, (GEN 1001, 20:63-21:2), which states: "[b]ecause the walls of the channels (*PDMS, not fluorinated*) and the carrier-fluid (fluorinated oil) are substantially different chemically...." (GEN1023, 1559.) No other discussion is provided in the specification or prosecution history regarding the term "non-fluorinated microchannel."

Applicant's statements during prosecution are the only indication of what Applicant understood to be the meaning of "non-fluorinated microchannel" and the Board should look to these statements in construing this term. *Advanced Fiber Technologies (AFT) Trust v. J & L Fiber Servs., Inc.*, 674 F.3d 1365, 1372 (Fed. Cir. 2012) ("A court should also consult the patent's prosecution history, which, like the specification, provides evidence of how the PTO and the inventor understood the claimed invention.").

The '083 patent lists polydimethylsiloxane (PDMS) as a preferred material for forming channels. (GEN1001, 15:60-62.) And dependent claim 14 recites that the channel is made of a polysiloxane. From the disclosure of the specification, and Applicant's statements during prosecution, a POSA would understand a "non-fluorinated microchannel" to be a microchannel made of a substance that does not contain a fluorine group in its chemical makeup, for example, a polysiloxane such

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

as PDMS. (GEN1002, ¶41.)

Fluorinated surfactant comprising a hydrophilic head group:

Surfactants concentrate at interfacial regions such as oil-water or liquid-solid interfaces. (GEN1022, 3; GEN1002, ¶42.) In systems such as the aqueous plugs in oil claimed in the '083 patent, a surfactant has a polar or ionic hydrophilic moiety and a nonpolar hydrophobic moiety, referred to as the head and tail groups, respectively. (GEN1022, 3; GEN1002, ¶42.) The '083 patent uses the term "surfactant" in the context of water-oil interfaces consistent with this definition: "In accordance with the devices and methods according to the invention, the surface chemistry to which solutions are exposed is preferably controlled through a careful selection of surfactants that are preferably designed to assemble at the interface between the plugs and the immiscible fluid that surrounds them." (GEN1001, 11:58-63.) Thus, a POSA would understand that the term "surfactant" as used in the '083 patent means a surface active agent with a hydrophilic head group and a hydrophobic tail group. (GEN1002, ¶42.)

A POSA would understand the term "comprising a hydrophilic head group" to not further limit the term "surfactant." (GEN1002, ¶43.) The term "surfactant" was amended to "fluorinated surfactant comprising a hydrophilic head group" during prosecution of the '083 patent in an amendment intended to overcome an obviousness rejection. (GEN1023, 1554-1558) The only mention of a hydrophilic

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

head group in the '083 patent is in discussing the preferred surfactant: "fluorinated surfactants, such as those with a hydrophilic head group, are preferred when the carrier-fluid is a fluorinated fluid and the plug-fluid is an aqueous solution." (GEN1001, 20:31-34.) But, as discussed above, a POSA would have understood that all surfactants have hydrophilic head groups, as a surfactant is defined as a molecule having a hydrophilic head group and a hydrophobic tail. (GEN1022, 3; GEN1002, ¶¶42-43.)

Surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface: Independent claims 1, 20 and 31 recite that the "fluorinated surfactant is present at a concentration such that the surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface." The '083 patent states, "when a fluorinated surfactant is introduced, the **surfactant reduces the surface tension at the oil-water interface preferentially over the wall-water interface.** This allows the formation of plugs that do not stick to the channel walls." (GEN1001, 20:65-21:2; emphasis added.) The claims of the '083 patent do not recite specific fluorinated surfactants or surfactant concentrations and the specification of the '083 patent does not discuss the use of specific fluorinated surfactants under specific conditions as being required to achieve a surface tension at the plug-fluid/microchannel interface that is higher than at the plug/carrier fluid

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

interface. (GEN1002, ¶44.) However, a POSA knows that if a sufficient concentration of surfactant is present such that the plugs flow smoothly without adhering to the channel walls, then the surface tension at the plug-wall interface will be higher than at the plug-carrier interface. (GEN1002, ¶44.) Thus, a POSA would understand that the claim term "surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface" merely represents a condition achieved when sufficient fluorinated surfactant is present in the carrier fluid such that the plugs do not adhere to the channel wall. (GEN1002, ¶44.)

Substantially encased/substantially surrounded on all sides: As discussed above, in defining "plugs," the '083 patent describes the interface between the aqueous plugs and the immiscible carrier fluid in terms of the plugs being "surrounded" by the carrier fluid: "wherein the aqueous plug-fluid is surrounded by a non-polar or hydrophobic fluid such as an oil." (GEN1001, 9:27-29 (emphasis added).) But the '083 patent also describes this same fluid interface in terms of the plugs being "encapsulated" by the carrier fluid:

In one aspect of the invention, the various devices and methods according to the invention are used to overcome one or more of the following problems involving microfluidics. First, the substantial dispersion of solutes in microfluidic channels increases reagent consumption and makes experiments or measurements over long time

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

scales (e.g., minutes to hours) difficult to perform. Various devices and methods according to the invention are intended to overcome this problem by localizing reagents *inside plugs that are encapsulated by an immiscible carrier-fluid.*

(*Id.*, 11:33 – 42 (emphasis added).)

Because the '083 patent uses the terms "surrounded" and "encapsulated" interchangeably when describing the fluid interface between the aqueous plugs and the immiscible carrier fluid, the BRI of "surrounded" includes "encapsulated." *See Solvay S.A. v. Honeywell Int'l, Inc.*, 622 F.3d 1367, 1380 (Fed. Cir. 2010) (holding that the claim term "isolating" can properly be construed to mean "separating" when the word "isolate" is used interchangeably with the word "separate" in the specification describing a chemical process); (GEN1002, ¶38).

And in construing a word of degree such as "substantially," it must be determined whether the patent provides "some standard for measuring that degree." *See Enzo Biochem, Inc. v. Applera Corp.*, 599 F.3d 1325, 1332 (Fed. Cir. 2010). Although the '083 patent contains no express disclosure for the term "substantially surrounded" or encapsulated in this context, it teaches that the invention is intended to overcome the problem of "dispersion of solutes in microfluidic channels [which] increases reagent consumption and makes experiments or measurements over long time scales (e.g., minutes to hours) difficult to perform." (GEN1001, 11:36-39.) The '083 patent overcomes this problem "by localizing reagents inside plugs that

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

are encapsulated by an immiscible carrier-fluid." (*Id.*, 11:41 – 42.) Thus, given that plugs have a cross-section that is substantially similar to the cross-section of the channels in which they are formed, a POSA would have understood that the BRI of "substantially surrounded" allows for plugs that are "entirely" surrounded or encapsulated by an immiscible carrier fluid such that a thin layer of the carrier fluid exists between the plugs and the channel walls. (GEN1002, ¶39.)

VI. Identification of challenge (37 C.F.R. § 42.104(b))

Petitioner requests IPR of all claims of the '083 patent. Per 37 C.F.R. § 42.6(c), copies of the references accompany the Petition. The Grounds for unpatentability are further supported by the accompanying declaration of Wilhelm Huck, Ph.D. ("Huck Dec.") (GEN1002), an expert in the fields of microfluidics and microscale reactions.

Ground	35 U.S.C. Section (pre-3/16/2013)	Claims	Index of References
1	§ 103(a)	1-31	Quake and Ramsey
2	§ 103(a)	18, 24	Quake, Ramsey and Green

Grounds 1 and 2 are not redundant. Ground 1 demonstrates that claims 1-31 would have been obvious to a POSA over the combination of Quake and Ramsey. But, if the Board were to find that the specific perfluorinated oils listed in claims 18 and 24 would not have been obvious over the combination of Quake and Ramsey, Ground 2 addresses the obviousness of claims 18 and 24 over the combination of Quake, Ramsey and Green.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Petitioner is reasonably likely to prevail in challenging the patentability of claims 1-31 on the basis of each ground herein.

A. Ground 1: Claims 1-31 would have been obvious over Quake and Ramsey

As supported by the declaration of Dr. Huck, claims 1-31 would have been obvious over U.S. Patent App. Pub. No. US 2002/0058332 ("Quake"; GEN1004) in combination with U.S. Patent 6,524,456 ("Ramsey"; GEN1006).

Quake qualifies as prior art under 35 U.S.C. § 102(e) as of September 14, 2001 and of September 15, 2000. First, Quake was filed by another on September 14, 2001, and published on May 16, 2002. Thus, Quake qualifies as prior art under 35 U.S.C. § 102(e) to the '083 patent claims as of its October 30, 2006 filing date.

Second, Quake claims priority to U.S. Prov. Appl. No. 60/233,037 ("the '037 application") (GEN1005), filed on September 15, 2000. The disclosures relied upon in Quake that teach the '083 patent claim limitations are also found in the '037 application and carry over into Quake. (GEN1002, ¶50.). Indeed, throughout this petition, Petitioner cites to both Quake and the '037 application to show where the '083 patent claim limitations are found in both. This evidences that the disclosures of Quake relied upon to demonstrate unpatentability of the '083 patent claims are fully supported in the '037 application. Thus, Quake also qualifies as prior art under 35 U.S.C. § 102(e) to the '083 patent claims as of the '037 application's filing date of September 15, 2000. *See In re Giacomini*, 612 F.3d

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

1380 (Fed. Cir. 2010); *Ex parte Yamaguchi*, 88 USPQ2d 1606 (BPAI 2008).

Ramsey was filed by another on September 29, 1999 and issued February 25, 2003. Thus, Ramsey qualifies as prior art under 35 U.S.C. § 102(e) to the '083 patent claims.

Claim 1	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
A microfluidic system comprising:	<p style="text-align: center;"><u>Quake</u></p> <p>"A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...)"(GEN1004, Abstract; <i>see</i> GEN1005, Abstract.)</p>
a non-fluorinated microchannel;	<p style="text-align: center;"><u>Quake</u></p> <p>"Channels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, of [sic] from silicon-urethane composites" (GEN1004, [0118]; GEN1005, 36:6-10.)</p> <p>"In a preferred embodiment, the invention provides a "T" on "Y" shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS." (GEN1004, [0216]; <i>see</i> GEN1005, 62:27-29.)</p>
a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel;	<p style="text-align: center;"><u>Quake</u></p> <p>"The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed." (GEN1004, ¶[0003]; <i>see</i> GEN1005, 1:14-17.)</p> <p>"In a preferred embodiment, water droplets are extruded into a flow of oil, but any fluid phase may be used as a droplet phase and any other incompatible or immiscible fluid or phase may be used as a barrier phase." (GEN1004, [0014]; <i>see</i> GEN1005, 6:14-16.)</p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils,"</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 1	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
	<p>(GEN1004, [0116-0117]; see GEN1005, 35:25-36:1.)</p> <p style="text-align: center;"><u>Ramsey</u></p> <p>"The segmenting fluid is preferably a liquid that is immiscible in the transport fluid and the reaction fluid(s).... Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required." (GEN1006, 6:36-50.)</p>
<p>at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid</p>	<p style="text-align: center;"><u>Quake</u></p> <p>"A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region." (GEN1004, Abstract; see GEN1005, Abstract.)</p> <p>"[T]he devices of this invention may be used to partition a first fluid into droplets within a second, incompatible or immiscible fluid." (GEN1004, ¶[0296]; see GEN1005, 82:2-3.)</p> <p>"In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa...." (GEN1004, ¶[00191]-[0092]; see GEN1005, 27:22-28:6.)</p> <p>"In embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous droplets are encapsulated or separated from each other by oil." (GEN1004, [0100]; see GEN1005, 30:2-3.)</p>
<p>wherein the fluorinated surfactant is present at a concentration such that surface tension</p>	<p style="text-align: center;"><u>Quake</u></p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils," (GEN1004, [0116-0117]; see GEN1005, 35:28-36:1.)</p> <p>"To prevent material (e.g., cells, virions and other</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 1	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.	particles or molecules) from adhering to the sides of the channels , the channels (and coverslip, if used) may have a coating which minimizes adhesion. ... Alternatively, the channels may be coated with a surfactant. " (GEN1004, [0094]) "The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane ... or fluorononane." (GEN1004, [0118]; <i>see</i> GEN1005, 36:8-10.)

As supported by the Huck declaration (GEN1002, ¶¶53-62), a POSA would have had a reason to modify the methods disclosed in Quake in view of Ramsey to arrive at the subject matter of claim 1. This is because Ramsey discloses that using fluorinated carrier oils in microfluidic systems where biocompatibility is required was already well-known in the art by 2002. And Quake teaches microfluidic systems for manipulating small reaction volumes (plugs) containing biological molecules which would benefit from the use of such a carrier oil. (GEN1004, ¶¶[0003], [0014], [0116]-[0117]; *see* GEN1005, 1:14-17, 6:14-16, 35:25-36:1; GEN1006, 6:36-50; GEN1002, ¶54.) The aqueous reaction volumes disclosed in Quake and Ramsey each include biological materials. (GEN1004, Abstract; *see* GEN1005, Abstract; GEN1006, Abstract; GEN1002, ¶54.) Ramsey explicitly teaches the use of fluorinated oils, perfluorocarbons in particular, in microfluidics systems because they are useful where biocompatibility is required. (GEN1006, 6:36-50; GEN1002, ¶54.) And Quake teaches that when doing biological reactions the reaction volume must contain a fluid that is physiologically compatible with

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

the biological material, including cells and virions. (GEN1004, [0116]; *see* GEN1005, 35:19-23; GEN1002, ¶54.) So, a POSA would have had a reason to use Ramsey's fluorinated oil, which Ramsey discloses as a good carrier for the biological molecules analyzed in the systems of Quake. (GEN1002, ¶54.) And a POSA would have had a reasonable expectation of success in so doing because Ramsey teaches that his fluorinated oil is a good carrier for biological molecules. (GEN1002, ¶54.)

Quake discloses a microfluidics system comprising a nonfluorinated microchannel, a carrier fluid oil comprising a fluorinated oil surfactant comprising a hydrophilic headgroup and one or more aqueous plugs substantially encased by the carrier fluid. (GEN1004, Abstract, [0100], [0114], [0117], [0216]; *see* GEN1005, Abstract, 30:2-3, 35:3-9, 38:28-36:1, 62:27-29; GEN1002, ¶55.) A POSA would have understood that Quake discloses plugs as recited in the claims, as Quake discloses that "droplets at these dimensions *tend to conform to the size and shape of the channels*, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa." (GEN1004, ¶¶[0091]-[0092] (emphasis added); *see* GEN1005, 28:4-6; GEN1002, ¶55.) While Quake does not explicitly disclose a carrier oil containing both a fluorinated oil and a fluorinated surfactant, Quake does disclose that the carrier oil may contain a fluorinated oil surfactant.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

(GEN1004, [0116-0117]; *see* GEN1005, 28:4-6; GEN1002, ¶56.) But a POSA would have had a reason to use a fluorinated oil in the systems of Quake from the teachings of Ramsey. (GEN1002, ¶56.)

Ramsey teaches microfluidics systems where aqueous droplets comprising a biological molecule are carried through microchannels in an immiscible "segmenting fluid." (GEN1006, 6:36-50; 3:62-67, 8:45-9:19; GEN1002, ¶57.) A POSA would have considered Ramsey's segmenting fluid to be a carrier fluid as recited in the '083 patent claims. (GEN1002, ¶57.) And Ramsey teaches that "the segmenting fluid should be biocompatible with [the] biological reagents that are used" in the reaction fluid. (GEN1006, 6:36-50; GEN1002, ¶57.) Ramsey teaches the use of fluorinated oils in microfluidics systems and provides a reason to use fluorinated carrier oils with biological materials, stating that "[p]erfluorocarbons [are] suitable because they are widely used where biocompatibility is required." (GEN1006, 6:36-50; GEN1002, ¶58.) And Quake teaches that when doing biological reactions the reaction volume must contain a fluid that is physiologically compatible with the biological material, including cells and virions. (GEN1004, [0116]; *see* GEN1005 35:22-23; GEN1002, ¶58.) So a POSA would have had a reason to ensure that the carrier fluid used in a microfluidic system, such as the one taught in Quake, was compatible with biological molecules as well. (GEN1002, ¶58.) Thus, a POSA using Quake's microfluidic system and looking for

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

physiologically compatible carrier oils would have had a reason to use Ramsey's fluorinated oil, which Ramsey discloses as a good carrier for the biological molecules analyzed in the systems of Quake. (GEN1002, ¶58.) And a POSA would have had a reasonable expectation of success in so doing because Ramsey teaches that his fluorinated oil is a good carrier for biological molecules. (GEN1002, ¶58.)

Surface tension effects: As shown in the claim chart above, claim 1 recites that the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface. As discussed in Section V, a POSA would understand that the claim term "surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface" represents a condition achieved when sufficient fluorinated surfactant is present in the carrier fluid such that the plugs do not adhere to the channel wall. (GEN1001, 20:65-21:2; GEN1002, ¶59.) Quake teaches such an addition of fluorinated surfactant. (GEN1002, ¶61.) A POSA would have understood that the claimed surface tension effects are obtained using a microfluidics system formed from the combination of Quake and Ramsey. (GEN1002, ¶61.)

As discussed by Dr. Huck, adhesion of plugs to the microchannel wall is prevented when the surface tension at the wall is greater than the surface tension at the fluid interface. These surface tension effects determine which fluid will wet the

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

microchannel wall. (GEN1002, ¶60.) When the surface tension is higher at the plug-fluid/wall interface than at the plug-fluid/carrier fluid interface, the plug-fluid will be repelled by the wall and will not wet, or adhere to, the wall. (GEN1001, 20:65-21:2; GEN1002, ¶60.)

The combination of Quake and Ramsey teaches how to avoid adhesion of plugs to the wall. Quake discloses use of fluorinated oils such as surfactants in carrier fluid and discloses use of such surfactants for coating the walls of microchannels. (GEN1004, [0094], [0117], [0118]; *see* GEN1005, 28:21-25; GEN1002, ¶61.) Quake also discloses that microchannel walls may be coated to prevent material from adhering to the microchannel. (GEN1004, [0094]; *see* GEN1005, 28:21-25; GEN1002, ¶61.) A POSA would have understood that Quake discloses the use of a fluorinated surfactant to prevent the plugs from sticking to microchannel walls. (GEN1002, ¶61.) Thus, a POSA would have understood that the combination of Quake and Ramsey discloses a microfluidics system where the surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface. (GEN1002, ¶61.)

A POSA would have had a reasonable expectation of successfully modifying Quake in view of Ramsey to arrive at the subject matter of claim 1, because this modification of Quake with Ramsey provides a microfluidics system where plugs do not adhere to the microchannel wall. (GEN1002, ¶62.) Claim 1 does not recite

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

specific fluorinated surfactants or surfactant concentrations. (GEN1002, ¶62.) And there is no disclosure in the '083 patent of specific fluorinated surfactants or specific surfactant concentrations required to achieve a surface tension at the plug-fluid/microchannel interface that is higher than at the plug/carrier fluid interface. (GEN1002, ¶62.)

As discussed above, a POSA would have understood that the combination of Quake and Ramsey provides motivation to achieve a higher surface tension at the plug-fluid/channel wall interface than at the plug-fluid/carrier interface. (GEN1002, ¶63.) This is because Quake teaches use of fluorinated surfactants for coating the channel wall to prevent material from adhering to the wall. (GEN1002, ¶63.) And, in general, a POSA has a reason not to want plugs to adhere to the channel wall, as this can cause cross-contamination between plugs. (GEN1004, [0008]; GEN1002, ¶63.) Thus, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 1 from the combination of Quake and Ramsey. (GEN1002, ¶63.)

Claim 20	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
A method of conducting a reaction within at least one plug, comprising the steps of:	<u>Quake</u> "Thus, individual particles or molecules can be separately compartmentalized inside individual droplets. These droplets can be analyzed, combined with other droplets (e.g. to react droplet contents) and/or sorted, as desired." (GEN1004, [0012]; <i>see</i> GEN1005, 5:20-23.)
introducing a carrier fluid comprising a	<u>Quake</u> "A microfluidic device provided by the invention

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 20	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
<p>fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device;</p>	<p>comprises a main channel and at least one inlet region which is in communication with the main channel at a droplet extrusion region." (GEN1004, ¶[0015]; <i>see</i> GEN1005, 7:6-10.)</p> <p>"The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed." (GEN1004, ¶[0003]; <i>see</i> GEN1005, 1:14-17.)</p> <p>"Channels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, of [sic] from silicon-urethane composites" (GEN1004, [0118]; <i>see</i> GEN1005, 36:6-10.)</p> <p>"In a preferred embodiment, the invention provides a "T" on "Y" shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS." (GEN1004, [0216]; <i>see</i> GEN1005, 62:27-29.)</p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils," (GEN1004, [0116-0117]; <i>see</i> GEN1005, 35:28-36:1.)</p> <p style="text-align: center;"><u>Ramsey</u></p> <p>"The segmenting fluid is preferably a liquid that is immiscible in the transport fluid and the reaction fluid(s).... Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required." (GEN1006, 6:36-50.)</p>
<p>introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the</p>	<p style="text-align: center;"><u>Quake</u></p> <p>"The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. ... By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that the</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 20	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
<p>carrier-fluid after the at least one stream contacts the carrier-fluid</p>	<p>stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets." (GEN1004, [0003]; <i>see</i> GEN1005, 1:14-2:3.)</p> <p>"[T]he devices of this invention may be used to partition a first fluid into droplets within a second, incompatible or immiscible fluid." (GEN1004, ¶[0296]; <i>see</i> GEN1005, 82:2-3.)</p> <p>"In preferred embodiments, the droplet extrusion region comprises a T-shaped junction between the inlet region and the main channel, so that the second fluid enters the main channel at an angle perpendicular to the flow of the first fluid, and is sheared off into the flow of the first fluid in the main channel." (GEN1004, ¶[0015]; <i>see</i> GEN1005, 7:7-10.)</p> <div data-bbox="597 924 1242 1285" data-label="Image"> </div> <p>FIG. 16A</p> <p>(GEN1004, Fig. 16A; <i>see</i> GEN1005, Fig. 16A.)</p> <p>In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa...." (GEN1004, ¶¶[00191]-[0092]; <i>see</i> GEN1005, 27:22-28:6.)</p>
<p>wherein the at least one plug-fluid comprises an aqueous fluid and at least one</p>	<p>Quake</p> <p>"A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ..." The</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 20	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
reagent for an autocatalytic reaction;	<p>microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region." (GEN1004, Abstract; <i>see</i> GEN1005, Abstract.)</p> <p>"[A] first fluid, which may be referred to as an 'extrusion' or 'barrier' fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a 'sample' or 'droplet' fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample." (GEN1004, ¶[0020]; <i>see</i> GEN1005, 8:14-18.)</p> <p>"Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR (13)" (GEN1004, [0080]; <i>see</i> GEN1005, 24:12-13.)</p>
the at least one plug-fluid is immiscible with the carrier-fluid;	<p style="text-align: center;"><u>Quake</u></p> <p>"[T]he devices of this invention may be used to partition a first fluid into droplets within a second, incompatible or immiscible fluid." (GEN1004, ¶[0296]; <i>see</i> GEN1005, 82:2-3.)</p> <p>"The fluid passing through the main channel and in which the droplets are formed is preferably one that is not miscible with the droplet forming fluid." (GEN1004, [0116]; <i>see</i> GEN1005, 35:23-25.)</p>
each plug is substantially surrounded on all sides by carrier-fluid;	<p style="text-align: center;"><u>Quake</u></p> <p>"In embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous droplets are encapsulated or separated from each other by oil." (GEN1004, [100]; <i>see</i> GEN1005, 30:2-3.)</p>
and the fluorinated surfactant is present at a concentration such that surface tension at the plug-	<p style="text-align: center;"><u>Quake</u></p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils," (GEN1004, [0116-0117]; <i>see</i></p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 20	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.	<p>GEN1005, 35:28-36:1.)</p> <p>"To prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels (and coverslip, if used) may have a coating which minimizes adhesion. ... Alternatively, the channels may be coated with a surfactant." (GEN1004, [0094].)</p> <p>"The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane ... or fluorononane." (GEN1004, [0118]; <i>see</i> GEN1005, 36:8-10.)</p>

Claim 20 is substantially similar to claim 1, but differs in that it (i) requires that the plug is "substantially surrounded on all sides" by the carrier oil instead of being "encapsulated" by the carrier oil as claim 1 requires; (ii) recites that plugs are formed in the carrier fluid; and (iii) recites that plug contains a reagent for an autocatalytic reaction. (GEN1001, 74:16-34.) Nonetheless, claim 20 would have been obvious over the combination of Quake and Ramsey.

As discussed for claim 1 above, Ramsey provides a biocompatible fluorinated oil for use in Quake's systems for analyzing biological molecules. (GEN1004, Abstract; *see* GEN1005, Abstract; GEN1006, Abstract, 3:62-4:48, 6:36-50; GEN1002, ¶65.) A POSA would have had a reason to combine the teachings of Quake and Ramsey to arrive at the subject matter of claim 20, because of these similarities in the systems of Quake and Ramsey, and because Quake teaches PCR, an autocatalytic reaction, while Ramsey teaches use of carriers that

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

do not interfere with biological molecules. (GEN1002, ¶65.) A POSA, wishing to perform PCR in a microfluidics system as taught by Quake would look to use the biocompatible fluorinated oils taught in Ramsey to prevent interference with the PCR reaction. (GEN1002, ¶65.)

As discussed in Section V, the BRI of "surrounded on all sides" by immiscible carrier fluid includes being "encapsulated" by carrier fluid to the extent that the carrier fluid forms a thin layer between the plug and the channel wall. (GEN1002, ¶66.) As shown above and discussed for claim 1, Quake teaches plugs that are "encapsulated" by the carrier oil. (GEN1002, ¶66.)

Claim 20 recites "introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid." As shown in the claim chart above, Quake teaches a continuous flow microfluidics system where plugs are formed by introducing aqueous solution containing samples into a pressurized stream of carrier oil. (GEN1004, [0003], Figure 16A; *see* GEN1005, 1:14-17, Figure 16A.; GEN1002, ¶67.)

Claim 20 also recites "wherein the at least one plug-fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction." As shown above, Quake teaches a microfluidics system for analysis of biological molecules. (GEN1004, Abstract; *see* GEN1005, Abstract; GEN1002, ¶68.) And Quake teaches

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

that PCR, an autocatalytic analysis of nucleic acid biological molecules, can be used in its microfluidics system. (GEN1004, Abstract, [0080]; *see* GEN1005, 24:12-13; GEN1002, ¶68.) A POSA would have understood that in order to perform PCR in a plug in a microfluidics system, the plug would need to contain the necessary reagents for the polymerase reactions which effect PCR analysis. (GEN1002, ¶68.)

Quake discloses the additional "introducing at least one stream of plug-fluid..." and "wherein the at least one plug-fluid comprises... at least one reagent for an autocatalytic reaction" limitations of claim 20 as shown in the claim chart, above. (GEN1004, [0003], Abstract, [0080], Figure 16A; *see* GEN1005, Abstract, 24:12-13, Figure 16A; GEN1002, ¶69.) And, as discussed for claim 1, a POSA would have had a reason to modify the systems of Quake to use the fluorinated carrier oil of Ramsey and would have understood that the systems of Quake achieve the claimed surface tension effects. (GEN1002, ¶69.) And a POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 20 from the teachings of Quake and Ramsey. This is because Quake teaches that autocatalytic reactions can be performed in microfluidics systems and Ramsey teaches the use of fluorinated carrier oils to avoid biocompatibility problems that can interfere with such autocatalytic reactions. (GEN1002, ¶69.)

Claim 31	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
-----------------	--

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 31	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
A microfluidic system comprising:	<p style="text-align: center;"><u>Quake</u></p> <p>"A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...)" (GEN1004, Abstract; <i>see</i> GEN1005, Abstract.)</p>
a non-fluorinated microchannel;	<p style="text-align: center;"><u>Quake</u></p> <p>"Channels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, of [sic] from silicon-urethane composites" (GEN1004, [0117]); <i>see</i> GEN1005, 36:6-10.)</p> <p>"In a preferred embodiment, the invention provides a "T" on "Y" shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS." (GEN1004, [0216]; <i>see</i> GEN1005, 62:27-29.)</p>
a fluorinated carrier fluid;	<p style="text-align: center;"><u>Quake</u></p> <p>"The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed." (GEN1004, ¶[0003]; <i>see</i> GEN1005, 1:14-17.)</p> <p>"In a preferred embodiment, water droplets are extruded into a flow of oil, but any fluid phase may be used as a droplet phase and any other incompatible or immiscible fluid or phase may be used as a barrier phase." (GEN1004, [0014]; <i>see</i> GEN1005, 6:14-16.)</p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils," (GEN1004, [0116-0117]; <i>see</i> GEN1005, 35:25-36:1.)</p> <p style="text-align: center;"><u>Ramsey</u></p> <p>"The segmenting fluid is preferably a liquid that is immiscible in the transport fluid and the reaction fluid(s).... Perfluorocarbons may also be suitable</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 31	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
	because they are widely used where biocompatibility is required." (GEN1006, 6:36-50.)
a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid;	<p style="text-align: center;"><u>Quake</u></p> <p>"The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ..., fluorinated oils, and other agents that are soluble in oil relative to water." (GEN1004, [0117]; <i>see</i> GEN1005, 35:28-36:1.)</p> <p>"To prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels (and coverslip, if used) may have a coating which minimizes adhesion. ... Alternatively, the channels may be coated with a surfactant." (GEN1004, [0094]; <i>see</i> GEN1005, 28:21-25.)</p> <p>"The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane ... or fluorononane." (GEN1004, [0118]; <i>see</i> GEN1005, 36:8-10.)</p> <p>"The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane." (GEN1004, ¶[0118]; <i>see</i> GEN1005, 36:8-10.)</p>
and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid	<p style="text-align: center;"><u>Quake</u></p> <p>"A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region." (GEN1004, Abstract; <i>see</i> GEN1005, Abstract.)</p> <p>"[T]he devices of this invention may be used to</p>

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Claim 31	Quake (GEN1004; GEN1005) and Ramsey (GEN1006)
	<p>partition a first fluid into droplets within a second, incompatible or immiscible fluid." (GEN1004, ¶[0296]; <i>see</i> GEN1005, 82:2-3.)</p> <p>"In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa...." (GEN1004, ¶¶[00191]-[0092]; <i>see</i> GEN1005, 27:22-28:6.)</p> <p>"In embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous droplets are encapsulated or separated from each other by oil." (GEN1004, [100]; <i>see</i> GEN1005, 30:2-3.)</p>
<p>wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.</p>	<p style="text-align: center;"><u>Quake</u></p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include ... fluorinated oils," (GEN1004, [0116-0117]; <i>see</i> GEN1005, 35:28-36:1.)</p> <p>"To prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels (and coverslip, if used) may have a coating which minimizes adhesion. ... Alternatively, the channels may be coated with a surfactant." (GEN1004, [0094]; <i>see</i> GEN1005, 28:21-25.)</p> <p>"The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane ... or fluorononane." (GEN1004, [0118]; <i>see</i> GEN1005, 36:8-10.)</p>

Claim 31 has the same limitations as claim 1, arranged in a slightly different way. As discussed for claim 1 above, both Quake and Ramsey disclose microfluidic systems using immiscible carrier fluids comprising surfactants as a

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

carrier fluid for an aqueous phase containing biological molecules. (GEN1004, Abstract; *see* GEN1005, Abstract; GEN1006, Abstract, 3:62-4:48; GEN1002, ¶70.) A POSA would have had a reason to combine the teachings of Quake and Ramsey to arrive at the subject matter of claim 30, because Ramsey provides a preferred biocompatible fluorinated oil for use in Quake's systems for analyzing biological molecules. (GEN1002, ¶70.) A POSA making a microfluidics system as taught by Quake would look to use the biocompatible fluorinated oils taught in Ramsey to use as a carrier for the aqueous plugs carrying biological molecules as taught in Quake. (GEN1002, ¶70.)

And a POSA would have had a reasonable expectation of successfully modifying Quake in view of Ramsey to arrive at the subject matter of claim 31, because this modification of Quake with Ramsey provides a microfluidics system where plugs do not adhere to the microchannel wall. (GEN1002, ¶71.) As discussed for claim 1 above, when the surface tension is higher at the plug-fluid/wall interface than the plug-fluid/carrier fluid interface, the plug-fluid will be repelled by the wall and will not wet, or adhere to, the wall. (GEN1002, ¶71.) The combination of Quake and Ramsey teaches how to avoid such adhesion. Quake discloses use of fluorinated oils such as surfactants in carrier fluid and discloses use of such surfactants for coating the walls of microchannels. (GEN1004, [0094], [0117], [0118]; *see* GEN1005, 28:21-25, 35:28-36:10; GEN1002, ¶71.)

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

The Board has found that the Examiner's actions do not inform the Board's decision on whether to institute *inter partes* review. *See, e.g., Butamax Advanced Biofuels LLC v GEVO Inc.*, IPR2013-00214, Paper 11 (Sept. 30, 2013). However, Petitioner directs the Board to the prosecution history of the '083 patent as the Examiner rejected the claims over Quake in view of Ramsey. (GEN1023, 1575-1577.) In response, applicants amended the claims to recite a non-fluorinated microchannel, and a fluorinated surfactant comprising a hydrophilic head group and generally argued that Quake and Ramsey do not teach these elements. (GEN1023, 1639-1640) The Examiner then allowed the case, stating that "[t]he prior art does not teach or fairly suggest the microfluidic system containing the particular fluorinated surfactant claimed." (GEN1023, 1657.) As shown in the discussion and charts above, and as supported by Dr. Huck, Quake and Ramsey do teach non-fluorinated microchannels, and do teach a fluorinated surfactant comprising a hydrophilic head group. (GEN1002, ¶¶54-56.)

For the above reasons, claims 1, 20 and 31 would have been obvious over the combination of Quake and Ramsey even in light of any allegations of objective indicia of non-obviousness. Objective indicia of non-obviousness are addressed with respect to all claims in Section C, below.

Claims 2 and 26: Claims 2 and 26 depend from claims 1 and 20, respectively, and recite that "the at least one plug contains at least one of a cell, a

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

virion, an enzyme, DNA, and RNA." Quake discloses "[a] microfluidic device for analyzing and/or sorting **biological materials** (e.g., molecules such as **polynucleotides and polypeptides, including proteins and enzymes; viruses and cells**) and methods for its use are provided.... Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region." (GEN1004, Abstract, emphasis added; *see* GEN1005, Abstract.) A POSA would have had a reason to practice the methods recited in claims 2 and 26, because Quake teaches that its microfluidics systems are useful for sorting and analyzing biological molecules and Ramsey teaches perfluorinated oils for carrying biological molecules. (GEN1002, ¶75.) A POSA would have also had a reasonable expectation of success in arriving at the subject matter of claims 2 and 26 from the combination of Quake and Ramsey as Quake discloses analysis of the same biological molecules as those recited in these claims and Ramsey discloses that fluorinated oils are best to use when biocompatibility is required. (GEN1002, ¶75.)

Claims 3, 4, 27 and 28: Claims 3 and 27 depend from claims 2 and 26, respectively, and recite that "the cell is a blood cell." Claims 4 and 28 depend from claims 2 and 26, respectively, and recite that "the cell is a bacterium." Quake discloses that "[t]he invention may be used to sort any prokaryotic (e.g., **bacteria**) or eukaryotic cells (e.g., mammalian, including human **blood cells**...." (GEN1004, [0117], emphasis added; *see* GEN1005, 52:6-8.) A POSA would have thus

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

understood that Quake discloses plugs containing a blood cell as recited in claims 3 and 4 and a bacterium as recited in claims 27 and 28, and would have understood that Ramsey discloses perfluorinated oils preferable for use with biological material such as blood cells. (GEN1002, ¶78.) Therefore, a POSA would have had a reason to practice the methods recited in claims 27 and 28. (GEN1002, ¶78.) Moreover, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 3, 4, 27 and 28 from the combination of Quake and Ramsey because Quake already demonstrated that blood and bacterium cells could be analyzed using microfluidics systems and Ramsey provided biocompatible perfluorinated oils for such analysis. (GEN1002, ¶78.)

Claims 5, 15 and 29: Claim 5 and 15 depend from claim 1. Claim 29 depends from claim 20.

Claim 5 recites that the microfluidic system of claim 1 further comprises a detection region. **Claim 15** recites that the microfluidic system of claim 1 further comprises at least one detector. Quake discloses that "[t]he device of the invention may also comprise a detection region which is within or coincident with at least a portion of the main channel... The device may also have **a detector**, ... associated with the detection region." (GEN1004, [0016]; *see* GEN1005, 7:11-14.)

Claims 5 and 29 recite determining whether "the contents of at least one plug have a selected characteristic." Quake discloses that "[b]iological material

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

within the droplets can be analyzed and/or **sorted by detecting a predetermined characteristic** of the biological sample in each droplet...." (GEN1004, Abstract, emphasis added; *see* GEN1005, Abstract.) A POSA would have understood that Quake discloses a microfluidic system having a detection region associated with a detector and discloses determining whether plugs containing biological materials have a selected characteristic. (GEN1002, ¶82.) And Ramsey discloses that microfluidics systems with fluorinated carrier oils are preferred for analysis of biological materials. As such, a POSA would have had a reason to practice the methods recited in claims 5, 15 and 29. (GEN1002, ¶82.) Moreover, Quake discloses a detection region and a detector, and discusses sorting plugs containing biological materials by detecting a predetermined characteristic. (GEN1004, Abstract, emphasis added; *see* GEN1005, Abstract.) And Ramsey discloses that fluorinated carrier oils are preferable when analyzing biological materials. (GEN1006, 6:36-50.) Thus, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 5, 15 and 29 from the combination of Quake and Ramsey. (GEN1002, ¶83.) This is because a POSA would have a reasonable expectation that the systems of Quake could be used for such sorting. (GEN1002, ¶83.)

Claim 16 depends from claim 15 and further requires that "the detector is selected from the group consisting of an optical detector, a fluorescence detector,

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

an electrochemical detector, a nuclear magnetic resonance detector, and a mass spectrometer detector." Quake discloses at least an optical detector: "[t]he device may also have a detector, **preferably an optical detector** such as a microscope, associated with the detection region." (GEN1004, [0016]; *see* GEN1005, 7:11-14; GEN1002, ¶84.) Quake also discloses detection of fluorescence using a microscope. (GEN1004, [0108]; *see* GEN1005, 33:12-14; GEN1002, ¶84.) Thus, a POSA would have understood that Quake discloses at least one of the detectors recited in claim 16, and therefore would have had a reason to use such a detector when practicing the methods taught in Quake in a system using the fluorinated oils of Ramsey to provide biocompatibility. (GEN1002, ¶84.) Moreover, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 16 from the combination of Quake and Ramsey as Quake already demonstrated that optical detectors could be successfully used with microfluidics systems for detecting biological material and Ramsey provides the preferable oils for use in analyzing biological material. (GEN1002, ¶85.)

Claims 6, 7, and 30: Claims 6 and 7 depend from claim 5, discussed above. Claim 30 depends from claim 29, discussed above.

Claim 6 recites the microfluidic system of claim 1 further comprising "a discrimination region of the system, in which the microchannel divides into at least a first and second branch, the discrimination region being downstream of the

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

detection region." **Claim 7** recites the system of claim 6, wherein "at least one plug is directed into one of the at least first and second branches of the microchannel in the discrimination region based on whether the contents of the at least one plug have the selected characteristic." **Claim 30** recites the method of claim 29, further comprising "directing the at least one plug into one of at least two branches of the microchannel based on whether the contents of the at least one plug have the selected characteristic." Thus, claims 6, 7 and 30 are drawn to microfluidic systems and methods where the system is divided into at least two branches and where a plug is directed into one of those branches based on a characteristic of a plug. Quake teaches such a microfluidics system. (GEN1002, ¶¶87-89.)

Quake discloses that "the device of the invention may also comprise a **discrimination region**, which is downstream from the detection region, and a flow control system that is responsive to the detector and **adapted to direct droplets through the discrimination region and into a branch channel**." (GEN1004, [0017], emphasis added; *see* GEN1005, 7:15-19.) The systems of Quake allow for the detection of selected characteristics of biological material in the plugs and the sorting of plugs into channels based on those characteristics. (*See* GEN1004, [0017]; *see* GEN1005, 7:15-19; GEN1002, ¶89.) And Rasmeý provides fluorinated carrier oils for use in analysis of biological material. (GEN1006, 6:36-50.) Thus, a POSA would understand that Quake teaches the embodiments of claims 6, 7 and

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

30, and a POSA would have had a reason to practice such embodiments. (GEN1002, ¶89.) Moreover, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 6, 7, and 30 from the combination of Quake and Ramsey as Quake already provided systems that could discriminate between plugs containing biological molecules and direct them to different channels based on specific characteristics and Ramsey already provided a fluorinated carrier oil for biocompatibility. (GEN1002, ¶89.)

Claim 8 depends from claim 6 and recites that the first branch of the microfluidic system differs from the second branch in at least one of "(a) diameter, (b) hydrophilicity, (c) net charge, (d) temperature, or (e) pressure." These methods for changing flow in a microfluidic system were well known in 2002. (GEN1002, ¶¶90-94.) Quake discloses that the "force and direction of flow can be controlled by any desired method for controlling flow, for example, by a **pressure differential**, by valve action or by **electro-osmotic flow**...." (GEN1004, [0125], emphasis added; *see* GEN1005, 38:16-18; GEN1002, ¶92.) Quake discloses that these methods of controlling flow "permit[] the movement of the cells into one or more desired branch channels or outlet regions." (GEN1004, [0125]; *see* GEN1005, 38:19-20; GEN1002, ¶92.) The discussions of Quake on sorting plugs are not limited to cells, but include "a mixture or population of molecule, cells or virions. (*See* GEN1004, [0125]-[0126]; *see* GEN1005, 38:13-29; GEN1002, ¶94.)

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

A POSA would have understood that Quake discloses a system of channels that differ in at least one of the characteristics recited in claim 8 and would have understood that these differences in characteristics could be used to direct flow in a microfluidics system. (GEN1002, ¶¶93-94.) And a POSA would have understood that the biocompatible fluorinated oils of Ramsey were a preferred choice for use in system analyzing biological materials. (GEN1002, ¶¶93-94.) Thus, a POSA would have had a reason to practice the methods recited in claim 8. Moreover, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 8 from the combination of Quake and Ramsey as Quake already demonstrated that plugs with biological material could be moved through microfluidics systems using the claimed methods for changing flow and Ramsey already provided a fluorinated carrier oil for biocompatibility. (GEN1002, ¶94.)

Claim 9 depends from claim 1 and recites that "the fluorinated surfactant comprises an oligoethylene glycol." Quake discloses that "non-limiting examples of nonionic surfactants which may be used include ... long chain carboxylic acid esters (for example, ... **polyoxyethylene glycol esters**, etc.)" (GEN1004, [0095], emphasis added.)

Applicants have not shown any criticality to the use of oligoethylene glycol surfactants. Instead, a POSA would have had a reason to use oligoethylene glycol surfactants from Quake's disclosure of polyethylene glycol surfactants, as a POSA

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

would have had a reasonable expectation that poloxyethylene glycol ester surfactants would have the same function as oligoethylene glycol surfactants. (GEN1002, ¶98.) Further, there is no discussion in the '083 patent suggesting that oligoethylene glycol surfactants have a different function than polyethylene glycol esters. (GEN1002, ¶98.) And a POSA would have had a reasonable expectation that the biocompatible fluorinated oils of Ramsey would work with the surfactants that Quake disclose can be used with biological materials. (GEN1002, ¶98.) Thus, a POSA would have had a reason to practice the embodiments of claim 9. (GEN1002, ¶98.) A POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 9 from the combination of Quake and Ramsey because Quake teaches using poloxyethylene glycol ester surfactants, which would have the same function as the oligoethylene glycol surfactant recited in claim 9, for use in systems analyzing biological materials. And Ramsey teaches the use of fluorinated carrier oil preferred for use with biological materials. (GEN1002, ¶98.)

Claims 10, 11 and 12: Claim 10 depends from claim 1 and recites that "the at least one plug contains at least one reagent for an autocatalytic reaction." Claims 11 and 21 depend from claims 10 and 20, respectively, and recite that "autocatalytic reaction is a polymerase-chain reaction." Thus, a POSA would understand that PCR is an autocatalytic reaction within the scope of claim 10.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

(GEN1002, ¶100.)

As discussed above for claim 20, Quake teaches analysis of biological molecules and teaches use of PCR in a microfluidics system and a POSA would understand that a PCR reagent would need to be added to the plug in order to perform PCR in the plug. (GEN1004, Abstract, [0080]; *see* GEN1005, Abstract, 24:12-13; GEN1002, ¶¶101, 105.) And Ramsey provides biocompatible fluorinated carrier oils for use in such systems. (GEN1006, 6:36-50.) Thus, a POSA would have had a reason to practice the embodiments of claim 10. And a POSA would have understood that Quake discloses microfluidics systems and methods performing PCR autocatalytic reactions, giving a POSA a reason to practice the embodiments of claims 11 and 21. (GEN1004, ¶[0002], [0080]; *see* GEN1005, 1:6-8, 24:12-13; GEN1017, 1047; GEN1002, ¶68, 101, 105.)

A POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 10, 11 and 21 from the combination of Quake and Ramsey, because Quake shows that PCR autocatalytic reactions of biological molecules can be performed in plugs in a microfluidics system and Ramsey provides preferred biocompatible fluorinated carrier oils. (GEN1002, ¶102.)

Claim 12 depends from claim 1 and recites that "the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters." Quake discloses that "[i]n preferred embodiments, the droplets of aqueous solution

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

have a **volume of approximately 0.1 to 100 picoliters** (pl)," which equates to a range of 100 femtoliters to 0.1 nanoliters. (GEN1004, ¶[0003]; *see* GEN1005, 2:1-3; GEN1002, ¶108.) As Quake teaches plugs containing biological materials with plug volumes falling in the middle of the claimed range, and as Ramsey teaches biocompatible fluorinated carrier oils, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claim 10 from the combination of Quake and Ramsey. (GEN1002, ¶108.) Further, Applicants have not shown any criticality to the plug volume recited in claim 12. "Where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *See In re Aller*, 220 F.2d 454, 456, (CCPA 1955); *see also In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003).

Claims 13 and 14: Claim 14 depends from claim 13 which depends from claim 1. Claim 13 recites that the "microchannel is made from a polymer, a glass or a metal," and claim 14 recites that "the polymer is a polysiloxane." Quake discloses working examples with "channels molded into optically transparent silicone rubber or **PolyDiMethylSiloxane (PDMS)**...." (GEN1004, [0216], emphasis added; *see* GEN1005, 62:27-29.) A POSA would have understood that PDMS is a polysiloxane polymer falling within the scope of claims 13 and 14. (GEN1002, ¶111.) A POSA would have understood that PDMS is a suitable material for use in

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

systems analyzing biological material and that PDMS is advantageous because microchannels can be fabricated into it using readily available semiconductor fabrication equipment. (GEN1004, [0216], emphasis added; *see* GEN1005, 62:27-63:15.) And a POSA would have understood from Ramsey that fluorinated carrier oils provide biocompatibility to such systems. (GEN1006, 6:36-50.) Thus, a POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 13 and 14 from the combination of Quake and Ramsey. (GEN1002, ¶111.)

Claims 17-18 and 22-24: Claim 17 depends from claim 1 and claim 18 depends from claim 17. Claim 22 depends from claim 20 and claims 23-24 depend from claim 22. Claim 22 recites the method of claim 20, "wherein the carrier fluid comprises a fluorinated compound."

Claims 17 and 23 recite that the fluorinated oil (claim 17) or fluorinated compound (claim 23) is perfluorinated. Claims 18 and 24 recite that the fluorinated oil (claim 18) or fluorinated compound (claim 24) "is selected from the group consisting of perfluorohexane, perfluoro(methylcyclohexane), perfluoro(1,3-dimethylcyclohexane), perfluorodecaline, perfluoroperhydrofluorene, perfluoroperhydrophenanthrene, perfluorotoluene, hexafluorobenzene, and combinations thereof." As discussed above for claims 1 and 20, Ramsey discloses a biocompatible perfluorinated oil and a POSA would have had a reason to use this

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

perfluorinated oil in the methods of Quake. (GEN1006, 6:36-50; GEN1002, ¶116.) Perfluorocarbons such as those recited in claims 18 and 24 were known to a POSA before May 9, 2002. (GEN1002, ¶116.) As such, Ramsey would have given a POSA a reason to use including at least one of the perfluorinated oils specified in claims 18 and 24, when using Quake's microfluidic device. (GEN1002, ¶116.) A POSA would also have had a reasonable expectation of success in arriving at the subject matter of claims 18 and 24 from the combination of Quake and Ramsey as the use of a perfluorinated oil in microfluidic devices such as Quake's device was known as evidenced by Ramsey. (GEN1002, ¶116.)

Claims 19 and 25 recite that the fluorinated oil (claim 19) or fluorinated compound (claim 25) "comprises a plurality of fluorinated substances of differing viscosity." Quake teaches that the "[p]eriodicity and droplet volume may also depend on channel diameter, **the viscosity of the fluids**, and shear pressure." (GEN1004, [0115]; *see* GEN1005, 35:17-18.) A POSA, looking to optimize the diameter of the droplets would understand from the teachings of Quake and Ramsey that fluorinated oils of different viscosities could be used. (GEN1002, ¶117.) *See Aller*, 220 F.2d at 456; *see also Peterson*, 315 F.3d at 1330. Further, as Quake teaches that viscosity is a parameter that can be modified to affect the droplet size, Quake would have demonstrated to a POSA that carrier fluid viscosity is a result effective variable that can be optimized. (GEN1002, ¶118.) The mere

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

optimization of a result-effective variable is insufficient to render a claim patentable. *See Peterson*, 315 F.3d at 1330. Therefore a POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 19 and 25 from the combination of Quake and Ramsey.

For the above reasons, claims 1-31 would have been obvious over the combination of Quake and Ramsey even in light of any allegations of objective indicia of non-obviousness. Objective indicia of non-obviousness are addressed with respect to all claims in Section C, below.

B. Ground 2: Claims 18 and 24 would have been obvious over Quake, Ramsey and Green

As supported by Dr. Huck, claims 18 and 24 would have been obvious over Quake and Ramsey in combination with "Perfluorocarbon Fluids," Chapter 4 by Green *et al.* in *Organofluorine Chemistry*, Banks *et al.*, eds., 1994 ("Green;" GEN1007). Quake and Ramsey are discussed in Ground 1. Green published in 1994 and qualifies as 35 U.S.C. §102(b) prior art to the '083 patent.

Claim 18 depends from claim 17 which depends from claim 1. Claim 24 depends from claim 22 which depends from claim 20. As discussed in Ground 1, claims 18 and 24 recite that the "fluorinated oil is selected from the group consisting of perfluorohexane, perfluoro(methylcyclohexane), perfluoro(1,3-dimethylcyclohexane), perfluorodecaline, perfluoroperhydrofluorene, perfluoroperhydrophenanthrene, perfluorotoluene, hexafluorobenzene, and

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

combinations thereof." These specific perfluorinated oils were well known in the art, as evidenced by Green.

Green summarizes the types of perfluorocarbons used in various applications before May 9, 2002. Green lists the commercially produced Flutec™ Fluids, including: perfluorohexane, perfluoro(methylcyclohexane), perfluorodecalin(*cis*- and *trans*- isomers), perfluoroperhydrofluorine, and perfluoroperhydrophenanthrene. (GEN1007, 4, Table 1.) This list includes five of the perfluorocarbons recited in claims 18 and 24. (GEN1002, ¶124.) Green also lists that perfluorinated oils can be used to lubricate surfaces. (GEN1007, 29.) A POSA would have understood that lubricants prevent adhesion to surfaces. (GEN1002, ¶124.) Green also discloses that "perfluorocarbons are virtually inert biologically...." (GEN1007, 28.)

A POSA would have had a reason to combine the teachings of Quake, Ramsey and Green, because a POSA using a perfluorinated oil taught by Ramsey in the systems of Quake would have looked to Green for more specific information on perfluorinated oils. (GEN1002, ¶125.) As discussed in Ground 1, a POSA would have had a reason to combine the teachings of Quake and Ramsey as both references are directed to microfluidic systems for manipulating small aqueous volumes (plugs) separated by oil carrier fluid. (GEN1004, Abstract; *see* GEN1005, Abstract; GEN1006, Abstract, 3:62-4:48; GEN1002, ¶125.) As discussed above,

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

Quake discloses using oils as carrier fluids and discloses use of fluorinated oils as surfactants in the carrier fluid oil in microfluidic systems. (GEN1004, ¶¶[0116-0117]; *see* GEN1005, 35:25-36:10; GEN1002, ¶125.) Ramsey discloses use of a perfluorinated oil as a carrier in a microfluidic system. and discloses that these perfluorinated oils are "widely used where biocompatibility is required." (GEN1006, 6:36-50.) Green is a chapter in a book on organofluorine chemistry that a POSA would look to for more information on perfluorinated oils. A POSA developing a microfluidics system from the teachings of Quake and Ramsey would have looked to Green for specific examples of Ramsey's perfluorinated oils. (GEN1002, ¶126.) Thus, a POSA would have had a reason to combine the teachings of Quake, Ramsey and Green. (GEN1002, ¶126.)

A POSA would have had a reasonable expectation of success in arriving at the subject matter of claims 18 and 24 from the teachings of Quake, Ramsey and Green. (GEN1002, ¶127.) As discussed in Ground 1, a POSA would have had a reasonable expectation of success in arriving at the subject matter of independent claims 1 and 20 from the teachings of Quake and Ramsey. (GEN1002, ¶127.) And a POSA, guided by Ramsey's teaching that perfluorocarbons are preferred for use with biological materials and Green's disclosure that perfluorocarbons are "virtually inert" to biological materials would have had a reasonable expectation that the perfluorocarbons listed in Green would function in the microfluidic

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

systems and methods recited in claims 18 and 24. (GEN1002, ¶127.)

For the above reasons, claims 18 and 24 would have been obvious over the combination of Quake, Ramsey and Green even in light of any allegations of objective indicia of non-obviousness. Objective indicia of non-obviousness are addressed with respect to all claims in Section C, below.

C. Objective indicia do not support patentability

In addition to Petitioner's strong *prima facie* obviousness showing outlined above, objective indicia must be taken into account, although they do not necessarily control the obviousness conclusion. *See Newell Cos., Inc. v. Kenney Mfg. Co.*, 864 F.2d 757, 768 (Fed. Cir. 1988). Objective evidence must be attributable to the claimed invention, and not attributable to what is unclaimed or present in the prior art. *In re Kao*, 639 F.3d 1057, 1068 (Fed. Cir. 2011) (finding that to be afforded substantial weight, objective indicia of non-obviousness must be tied to the novel elements of the claim at issue.) Even when there is substantial evidence of relevant objective indicia, such evidence may be inadequate to overcome a strong *prima facie* obviousness. *See, e.g., Leapfrog Enterprises Inc. v. Fisher-Price Inc.*, 485 F.3d 1157, 1162 (Fed. Cir. 2007).

Patent Owner raised no objective indicia of non-obviousness during prosecution of the '083 patent. (GEN1023.) To the extent Patent Owner may now argue that objective indicia exists based on acquiescence by licensing, commercial

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

success, long-felt but unmet need, failure of others, industry praise, or unexpected superior results, any such arguments are unsupported by the available evidence and fail to weigh in favor of patentability in view of Petitioner's strong *prima facie* showing of obviousness. Also, Petitioner should be given an opportunity to rebut any objective indicia of non-obviousness that Patent Owner raises for the first time in this proceeding or in the parallel district court litigation.

1. No acquiescence by licensing

Licensing can evidence non-obviousness where (1) a nexus between the merits of the invention and the licenses exists and (2) proof exists that the license arose out of substantial industry recognition and acceptance of the patent's validity. *See Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530 (Fed. Cir. 1983) ("[Patent Owner] has shown neither a nexus between the merits of the invention and the licenses of record, nor that those licenses arose out of recognition and acceptance of the patent"); *see also Santarus, Inc. v. Par Pharm., Inc.*, 720 F. Supp. 2d 427, 459 (D. Del. 2010), *aff'd in relevant part* 694 F.3d 1344, 1357 (Fed. Cir. 2012). And when licensing a patent is in the economic interest of the licensee, regardless of the validity of the patent, then the license does not suggest non-obviousness. *See EWP Corp. v. Reliance Universal Inc.*, 755 F.2d 898, 907-08 (Fed. Cir. 1985) ("[Licensing] programs are not infallible guides to patentability. They sometimes succeed because they are mutually beneficial to the licensed group ... or for other

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

reasons unrelated to the unobviousness of the licensed subject matter.").

Patent Owner has exclusively licensed the '083 patent to RainDance Technologies, Inc. ("RainDance") (GEN1019, ¶¶3-4, 50.) However, there is no available evidence to suggest that RainDance entered the licensing agreement, not merely for RainDance's economic interests, but instead in acquiescence to the merits of the alleged invention. Moreover, as RainDance is the only purported licensee of the '083 patent, there is no evidence to suggest widespread industry recognition and acceptance of the '083 patent through licensing by parties other than RainDance. Therefore, the available evidence does not show that acquiescence by licensing supports the patentability of the '083 patent claims.

2. No commercial success

Patent Owner may assert that RainDance's RainDrop[®] system is a commercial embodiment of the '083 patent. As an initial matter, no available evidence demonstrates that the RainDrop[®] system either (i) practices the features claimed in the '083 patent or (ii) has substantially increased in market share, for example, by substantially expanding the market or by substantially displacing competing products in the market. But even if the RainDrop[®] system practices the features claimed in the '083 patent, any alleged commercial success of the system would be attributable to features that were present in the prior art. (GEN1002, ¶120.) For example, Quake discloses isolating single DNA molecules in an

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

aqueous droplet in a microfluidic system. (*See, e.g.*, GEN1004, ¶¶[0015], [0020], [0120]; GEN1005, 6:29-7:1; GEN1002, ¶120.) And a POSA would have understood that conducting reactions, such as PCR, in microfluidic systems using small volumes of aqueous fluid surrounded by a carrier fluid was well-known in the art before May 2002. (*See, e.g.*, GEN1004, ¶¶[0018], [0080], [0317], [0318]; GEN1005, 8:14-9:2, 24:12-13, 85:12-25; GEN1006, 8:45-9:63; GEN1011, pp. 4-10; GEN1002, ¶120.) Therefore, the available evidence does not show that any alleged commercial success of the RainDrop[®] system—or any other product—supports the patentability of the '083 patent claims. *Dippin' Dots, Inc. v. Mosey*, 476 F.3d 1337, 1345 (Fed. Cir. 2007) (where a product's commercial success can be attributed to characteristics of the invention that were already in the prior art, non-obviousness is not shown.)

3. No long-felt but unmet need or failure of others

A showing of a long-felt and unmet need requires that the need must have been a persistent one that was recognized by those of ordinary skill in the art. *Ecolochem, Inc. v. S. Cal. Edison Co.*, 227 F.3d 1361, 1377 (Fed. Cir. 2000). Also, the long-felt need must not have been satisfied by another before the invention. *Minn. Mining & Mfg. Co. v. Johnson & Johnson Orthopedics, Inc.*, 976 F.2d 1559, 1574-75 (Fed. Cir. 1992). And the invention must in fact satisfy the long-felt need. *Id.* at 1575. Failure of others to find a solution to the problem which the patent

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

purports to solve is also relevant in determining non-obviousness. *Id.*

There was no long-felt but unmet need or failure of others to make a microfluidic device to prevent cross-contamination between plugs by May 2002. As discussed in § IV.A.2. above, microfluidic devices existed before May 2002. (GEN1002, ¶122.) And devices for forming stable, aqueous plugs that do not adhere to the system wall were also known at that time. (GEN1004; GEN1005; GEN1010; GEN1018; GEN1011; GEN1002, ¶122.) There is no available evidence that these prior art devices failed to satisfy a need or solve a problem that was allegedly met or solved by the features claimed in the '083 patent. (GEN1002, ¶122.) Therefore, the available evidence does not show that a long-felt but unmet need or a failure of others supports the patentability of the '083 patent claims.

4. No industry praise

Praise for an invention by peers in the industry may support non-obviousness, but only if such praise is directed to claimed features of the invention and not to elements found in the prior art. *PharmaStem Therapeutics, Inc. v. ViaCell, Inc.*, 491 F.3d 1342, 1365-66 (Fed. Cir. 2007).

Although the RainDrop[®] system was named among the "Top Ten Innovations of 2014" by the magazine *The Scientist*, there is no evidence that the award is attributable to the methods recited in the '083 patent claims. (GEN1020.) And to the extent that Patent Owner contends that this recognition is attributable to

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

features claimed in the '083 patent, these features were taught in the prior art. (*See, e.g.,* GEN1004, ¶[0013]; GEN1005, 5:19-23; GEN1011, pp. 4-10; GEN1002 ¶124.) As such, this recognition by *The Scientist* should be given no weight in a nonobviousness analysis. *In re Kao*, 639 F.3d at 1068. And Petitioner is not aware of any other evidence of praise in the industry that weighs in favor of patentability. (GEN1002, ¶125.) Therefore, the available evidence does not show that industry praise supports the patentability of the '083 patent claims.

5. No unexpectedly superior results

Non-obviousness of an invention can be demonstrated by showing that the claimed invention exhibits a superior property or advantage, *i.e.*, unexpectedly superior results, over the closest prior art. *In re De Blauwe*, 736 F.2d 699, 705 (Fed. Cir. 1984) Unexpectedly superior results must be commensurate in scope with the claims. *In re Boesch*, 617 F. 2d 272, 277 (C.C.P.A. 1980); *In re Peterson*, 315 F.3d 1325, 1329-31 (Fed. Cir. 2003).

Patent Owner may allege that the RainDrop[®] system's performance is unexpectedly superior compared to that of prior art microfluidic systems, and that such unexpectedly superior performance is attributable to alleged inventions claimed in the '083 patent. But as discussed above, the prior art disclosed isolating single DNA molecules in an aqueous plug and conducting reactions, such as PCR, in microfluidic systems using small volumes of aqueous fluid surrounded by a

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

carrier. (*See, e.g.*, GEN1004, ¶¶[0018], [0015], [0317], [0318]; GEN1006, 8:45-9:63; GEN1011, pp. 4-6; GEN1002, ¶128.) As such, no available evidence demonstrates that the RainDrop[®] system uses features claimed in the '083 patent to perform these same steps with unexpectedly superior results compared to those obtained using the devices and methods disclosed in the prior art. (GEN1002, ¶128.) Therefore, the available evidence does not show that unexpected superior results support the patentability of the '083 patent claims.

VII. Conclusion

Each of claims 1-31 would have been obvious over the art discussed above. Each of Grounds 1-2 amply demonstrates that a POSA would have arrived at claims 1-31 with a reasonable expectation of success. Indeed, claims 1-31 are no more than a combination of well-known prior art elements that yield predictable results. And nothing in claims 1-31 would have required an advancement over known methods. Moreover, no objective indicia of nonobviousness weigh in favor of patentability. As such, the Board should institute IPR for each challenged claim.

VIII. Mandatory notices (37 C.F.R. § 42.8(a)(1))

Real Party-In-Interest (37 C.F.R. § 42.8(b)(1)): 10X Genomics, Inc.

Related Matters (37 C.F.R. § 42.8(b)(2)): **Judicial:** *RainDance Techs., Inc., et al. v. 10X Genomics, Inc.*, 1:15-cv-00152-RGA (D. Del. Feb. 2, 2015).

Administrative: Petitioners are concurrently filing petitions for IPR of U.S. Patent Nos. 8,822,148; 8,273,573; 8,304,193 and 8,329,407.

Petition for Inter Partes Review of U.S. Patent No. 8,889,083**Designation of Lead and Back-Up Counsel (37 C.F.R. § 42.8(b)(3)):**

Lead Counsel	Back-Up Counsel
Eldora L. Ellison (Reg. # 39,967) STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C. 1100 New York Avenue, NW Washington, DC 20005 202.772.8508 (telephone) 202.371.2540 (facsimile) eellison-PTAB@skgf.com	Deborah A. Sterling (Reg. # 62,732) STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C. 1100 New York Avenue, NW Washington, DC 20005 202.772.8501 (telephone) 202.371.2540 (facsimile) dsterlin-PTAB@skgf.com

Notice of Service Information (§ 42.8(b)(4)): Please direct all correspondence to lead and back-up counsel at the above address. Petitioner consents to email service at: eellison-PTAB@skgf.com and dsterlin-PTAB@skgf.com.

This Petition is filed in accordance with 37 C.F.R. § 42.106(a). Concurrently filed are a Power of Attorney and Exhibit List under 37 C.F.R. § 42.10(b) and § 42.63(e), respectively. The required fee is paid through online credit card payment. The Office is authorized to charge any fee deficiency, or credit any overpayment, to Deposit Acct. No. 19-0036 (Customer ID No. 45324).

Respectfully submitted,
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/Eldora L. Ellison/
Eldora L. Ellison
Registration No. 39,967
Attorney for Petitioner

Date: May 6, 2015
1100 New York Avenue, N.W.
Washington, D.C. 20005
(202) 371-2600

1985106_1

Petition for Inter Partes Review of U.S. Patent No. 8,889,083

CERTIFICATION OF SERVICE (37 C.F.R. §§ 42.6(e), 42.105(a))

The undersigned hereby certifies that the above-captioned "Petition for *Inter Partes* Review of U.S. Patent No. 8,889,083 under 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42.1-.80, 42.100-.123," including its supporting evidence (Exhibits 1001-10XX), was served in its entirety on May 6, 2015, upon the following parties via FedEx:

Brown Rudnick
One Financial Center
Boston, MA 02111

*Patent owner's correspondence
address of record for U.S. Patent No.
8,889,083*

Edward R. Reines
Sonal N. Mehta
Derek C. Walter
Blake Davis
WEIL, GOTSHAL & MANGES LLP
201 Redwood Shores Parkway
Redwood Shores, CA 94065
*Additional address known to
Petitioner as likely to effect service*

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Date: May 6, 2015
1100 New York Avenue, N.W.
Washington, D.C. 20005
(202) 371-2600

/Eldora L. Ellison/
Eldora L. Ellison
Registration No. 39,967
Attorney for Petitioner

EXHIBIT C

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BIO-RAD LABORATORIES, INC. and
THE UNIVERSITY OF CHICAGO,

Plaintiffs,

v.

10X GENOMICS, INC.,

Defendant.

C.A. No. 15-152-RGA

**CONTAINS OUTSIDE ATTORNEYS'
EYES ONLY INFORMATION**

**EXPERT REPORT OF PROFESSOR HSUEH-CHIA CHANG
REGARDING THE INVALIDITY OF U.S. PATENT NOS.
8,329,407; 8,304,193; 8,822,148; 7,129,091; AND 8,889,083**

Of Counsel:

David I. Gindler
Andrei Iancu
Lauren N. Drake
Elizabeth C. Tuan
IRELL & MANELLA LLP
1800 Avenue of the Stars, Suite 900
Los Angeles, CA 90067-4276

Michael H. Strub
Dennis J. Courtney
IRELL & MANELLA LLP
840 Newport Center Drive, Suite 400
Newport Beach, CA 92660
(949) 760-0991

RICHARDS, LAYTON & FINGER, P.A.
Frederick L. Cottrell, III (#2555)
Jason J. Rawnsley (#5379)
920 North King Street
Wilmington, DE 19801
(302) 651-7700
cottrell@rlf.com
rawnsley@rlf.com

Attorneys for 10X Genomics, Inc.

Dated: August 21, 2017

TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION	1
II. BACKGROUND AND QUALIFICATIONS	2
III. BASES OF OPINIONS AND MATERIALS CONSIDERED	4
IV. SUMMARY OF OPINIONS	4
V. LEGAL STANDARDS	6
A. Claim Construction	6
B. Invalidity Legal Standards	6
1. Prior Art	7
2. Anticipation.....	7
3. Obviousness	8
4. Written Description Requirement	11
5. Enablement	11
6. Indefiniteness	12
7. Conception and Reduction to Practice.....	12
8. Level of Ordinary Skill in the Art.....	13
VI. PRINCIPLES AND BACKGROUND KNOWLEDGE IN THE PRIOR ART	14
A. Microfluidic Systems and Plugs	14
1. Single Phase Systems.....	15
2. Two Phase Systems.....	15
(a) Slug Systems	15
(b) Droplet Systems	17
B. Microfluidic Systems and Conducting Reactions	26
C. Carrier Fluid.....	35

	<u>Page</u>
D. Two or More Aqueous Fluids	38
E. Bio-Rad’s View of the State of the Art	39
VII. THE ASSERTED PATENTS	42
A. Background	42
1. ’407 Patent	42
2. ’193 Patent	43
3. ’148 Patent	44
4. ’091 Patent	44
5. ’083 Patent	45
B. Claim Construction	46
VIII. PRIOR ART	48
A. Quake	48
B. Shaw Stewart	56
C. Burns (2001)	58
D. Nisisako	60
E. Thorsen	62
F. Seki	63
G. Corbett	64
H. Lagally	65
I. Burns (1996)	66
J. Curcio	67
K. Anderson	70
L. Wang	73
M. Ramsey	74

	<u>Page</u>
N. Schubert	75
O. Krafft.....	75
P. Kenis	76
Q. Weigl.....	77
R. Kopf-Sill	78
S. Erbacher	80
T. Whitesides.....	80
IX. INVALIDITY OF THE '407 PATENT.....	82
A. Summary of the '407 Patent	82
1. Priority	82
B. Invalidity Overview	89
C. Invalidity Under 35 U.S.C. § 112	90
1. Written Description.....	90
2. Enablement	101
3. Indefiniteness	119
D. Invalidity Based on Prior Art.....	121
1. Anticipation.....	121
(a) Invalidity Based on Quake.....	121
(b) Invalidity Based on Shaw Stewart	137
2. Obviousness	149
(a) Invalidity Based on Quake.....	149
(b) Invalidity Based on Shaw Stewart	188
(c) Invalidity Based on Burns (2001)	227
(d) Invalidity Based on Nisisako	266

	<u>Page</u>
(e) Invalidation Based on Thorsen	302
(f) Invalidation Based on Seki	338
3. Motivation to Combine and Reasonable Expectation of Success	375
X. INVALIDITY OF THE '193 PATENT	389
A. Summary of the '193 Patent	389
1. Priority	389
B. Invalidation Overview	398
C. Invalidation Under 35 U.S.C. § 112	399
1. Written Description	399
2. Enablement	410
3. Indefiniteness	427
D. Invalidation Based on Prior Art	430
1. Obviousness	430
(a) Invalidation Based on Quake	430
(b) Invalidation Based on Shaw Stewart	465
(c) Invalidation Based on Burns (2001)	498
(d) Invalidation Based on Nisisako	530
(e) Invalidation Based on Thorsen	559
(f) Invalidation Based on Seki	590
2. Motivation to Combine and Reasonable Expectation of Success	620
XI. INVALIDITY OF THE '148 PATENT	633
A. Summary of the '148 Patent	633
1. Priority	633
B. Invalidation Overview	636

	<u>Page</u>
C. Invalidity Under 35 U.S.C. § 112	637
1. Written Description	637
2. Enablement	649
3. Indefiniteness	668
D. Invalidity Based on Prior Art	669
1. Obviousness	669
(a) Invalidity Based on Quake	669
(b) Invalidity Based on Shaw Stewart	702
(c) Invalidity Based on Burns (2001)	732
(d) Invalidity Based on Nisisako	762
(e) Invalidity Based on Thorsen	789
(f) Invalidity Based on Seki	816
2. Motivation to Combine and Reasonable Expectation of Success	844
XII. INVALIDITY OF THE '091 PATENT	857
A. Summary of the '091 Patent	857
1. Priority	857
B. Invalidity Overview	862
C. Invalidity Under 35 U.S.C. § 112	863
1. Written Description	863
2. Enablement	870
D. Invalidity Based on Prior Art	886
1. Obviousness	886
(a) Invalidity Based on Quake	886
(b) Invalidity Based on Shaw Stewart	959

	<u>Page</u>
(c) Invalidity Based on Burns (2001)	1024
(d) Invalidity Based on Nisisako	1100
(e) Invalidity Based on Thorsen	1167
(f) Invalidity Based on Seki	1231
2. Motivation to Combine and Reasonable Expectation of Success.....	1293
XIII. INVALIDITY OF THE '083 PATENT	1307
A. Summary of the '083 Patent	1307
1. Priority	1307
B. Invalidity Overview	1312
C. Invalidity Under 35 U.S.C. § 112	1313
1. Written Description.....	1313
2. Enablement	1320
3. Indefiniteness	1335
D. Invalidity Based on Prior Art.....	1337
1. Obviousness	1337
(a) Invalidity Based on Quake.....	1337
(b) Invalidity Based on Shaw Stewart	1389
(c) Invalidity Based on Burns (2001)	1439
(d) Invalidity Based on Nisisako	1489
(e) Invalidity Based on Thorsen	1536
(f) Invalidity Based on Seki	1585
2. Motivation to Combine and Reasonable Expectation of Success.....	1631
XIV. SECONDARY CONSIDERATIONS	1643
A. Praise of Others.....	1644

	<u>Page</u>
B. Long Felt But Unresolved Need	1649
C. Failure of Others	1650
D. Commercial Success	1652
E. Skepticism By Experts.....	1653
F. Teaching Away By Others.....	1653
G. Recognition of a Problem	1654
H. Copying.....	1654
I. Unexpected Results.....	1654
J. Simultaneous Invention	1655
XV. CONCLUSION.....	1659

I. INTRODUCTION

1. I have been retained as an independent expert witness in this lawsuit on behalf of 10X Genomics, Inc. (“10X”) to testify as a technical expert concerning the technology at issue in U.S. Patent Nos. 8,329,407 (the “’407 patent”), 8,304,193 (the “’193 patent”), 8,822,148 (the “’148 patent”), 7,129,091 (the “’091 patent”), and 8,889,083 (the “’083 patent”) (collectively, the “Ismagilov patents”).

2. I understand that Bio-Rad Laboratories, Inc. and The University of Chicago (collectively, “Bio-Rad” or “Plaintiffs”) have sued 10X claiming infringement of the Ismagilov patents. In this expert report, I provide opinions on the general subject matter of the Ismagilov patents, as well as my opinion that the asserted claims of the Ismagilov patents are invalid, among other issues. I submit this expert report in compliance with Rule 26(a)(2) of the Federal Rules of Civil Procedure on issues pertaining to the technologies of the Ismagilov patents, and in particular regarding prior art relevant to the Ismagilov patents as set forth in this expert report and in any supplemental reports or declarations that I may prepare for this litigation in the future. I may also provide testimony in the form of a tutorial on the technology relating to the Ismagilov patents and the significance and meaning of these patents in the context of Bio-Rad’s allegations and the relevant technological background. I also expect to testify at trial with respect to the subject matter of my report and matters addressed by any expert testifying on behalf of Bio-Rad if asked about these matters by the Court or by the parties’ counsel. I may also testify on other matters relevant to this case if asked by the Court or by the parties’ counsel.

3. I am being compensated for my work on this matter at my standard consulting rate of \$400 per hour. My compensation does not depend in any way on the outcome of this case or any other case.

4. I reserve the right to supplement or amend this report as appropriate, including if

additional facts and information that affect my opinions become available.

II. BACKGROUND AND QUALIFICATIONS

5. I am currently Bayer Professor of Chemical Engineering at the University of Notre Dame and Director of the University of Notre Dame's Center for Microfluidics and Medical Diagnostics. I have been a professor of Chemical Engineering at the University of Notre Dame since 1987, and Director of the Center for Microfluidics and Medical Diagnostics since 2003.

6. I received my Bachelor of Science degree in Chemical Engineering from the California Institute of Technology in 1976, and a Ph.D. in Chemical Engineering from Princeton University in 1980. From 1980 to 1984, I was an Assistant and then an Associate Professor at the University of California, Santa Barbara. From 1984 to 1987, I was an Associate Professor at the University of Houston. In addition to being a Professor at the University of Notre Dame, I have also been an Adjunct Professor at National Tsing Hua University, an Adjunct Professor at National Cheng Kung, a Distinguished Visiting Fellow at Imperial College, and Senior Visitor in the Department of Applied Mathematics and Theoretical Physics at the University of Cambridge.

7. I have extensive experience in the field of microfluidics. My current research includes the refinement of high-throughput methods of generating droplets of water solutions in oil with monodisperse size at the submicron or micron scale, in which reactions involving nucleic acids may be conducted. I have extensive knowledge of the science and technology underlying the patents in this litigation. By way of example only, I have worked on and studied droplet transport in both square and circular microchannels, the dispersion of reactants in microchannels, chaotic mixing in droplets, droplet generation, and nucleic acid quantification in droplets.

8. I am Chief and Founding Editor of *Biomicrofluidics* (founded 2006), and have

also served on the editorial board of *Membrane* since 2011. From 2000 to 2009, I served as an Associate Editor of *SIAM Journal of Applied Mathematics*, and from 1990 to 1995 I served on the *Editorial Board of International Journal of Bifurcation and Chaos in Applied Sciences and Engineering*.

9. I am the author or co-author of over 250 scientific papers in the fields of microfluidics, reaction engineering, fluid mechanics, and biosensing. These papers set forth my numerous contributions to the field of microfluidics. For example, I am one of the first researchers to study droplet transport in square and circular microchannels and to study the effect of surfactants on the droplet transport. Ratulowski, J. and Chang, H.-C., "Transport of gas bubbles in capillaries," *Phys of Fluids*, 1:1642-1655 (1989) ("Ratulowski") (10X-000251076-90); Takhistov, P., Indeikina A. and Chang, H.-C., "Electrokinetic displacement of air bubbles in microchannels," *Phys of Fluids*, 14: 1-14 (2002) (10X-000255671-84). I also developed a theory for characterizing analyte dispersion due the shear flow. Balakotaiah, V. and Chang, H.-C., "Dispersion of Chemical Solutes in Chromatographs and Reactors," *Phil. Trans of the Royal Society of London*, A351:39-75 (1995) (10X-000255881-917). I also developed AC electrospray and surface acoustic wave technologies for microdroplet and nanodroplet generation for mass spectrometry. Yeo, L., Lastochkin, D., Wang, S.-C. and Chang, H.-C., "A New ac Electrospray Mechanism by Maxwell-Wagner Polarization and Capillary Resonance," *Physical Review Letters*, 92:133902-133904 (2004) (10X-000256222-25); Ho, J., Tan, M. K., Go, D. B., Friend, J. R. and Chang, H.-C., "A Paper-Based Microfluidic Surface Acoustic Wave Sample Delivery and Ionization Source for Rapid and Sensitive Ambient Mass Spectrometry," *Anal. Chem.*, 83:3260-3266 (2011) (10X-000255243-49). I have also done extensive work relating to nucleic acid detection and quantification. Taller, D., Richards K., Slouka, S., Senapati, S., Hill, R., Go, D. B.

and Chang, H.-C., “On-chip surface acoustic wave lysis and ion-exchange membrane detection of exosomal RNA for pancreatic cancer study and diagnosis,” *Lab Chip*, 15:1656-1666 (2015) (10X-000255941-52).

10. I have not testified as an expert at trial or by deposition.

11. Further details of my background and experience, including a list of my publications, are provided in my curriculum vitae, which is attached as **Exhibit 1**.

III. BASES OF OPINIONS AND MATERIALS CONSIDERED

12. In forming my opinions in this Expert Report and for expert testimony that I may be called upon to provide, I have considered and may rely on at least the documents identified in this report or identified in **Exhibit 2** attached hereto. This includes, but is not limited to: the Ismagilov patents and related prosecution histories, patent contentions submitted by the parties (including documents referred to in those contentions), prior art references, publicly available information regarding the patented subject matter, third-party information, deposition testimony, documents produced in this action, and discovery responses, among other materials. My opinion is based on my review of such materials, together with my education, training, and experience in the relevant field.

13. In testifying, and to support or summarize my opinion, I may use some or all of the documents and information identified in this report or identified in **Exhibit 2**, additional information identified in discovery, as well as any materials relied upon by Plaintiffs’ experts. In addition, I may supplement these materials with other materials, charts, illustrations, or diagrams to provide additional context, background, or information, and may prepare summaries and demonstrative exhibits (such as a PowerPoint presentation or live demonstration) to assist my presentation of testimony to the Court.

IV. SUMMARY OF OPINIONS

14. Based on my investigation and preparation in connection with this matter and my prior experience in the field, I have developed an understanding of the alleged inventions described and claimed in the Ismagilov patents. In particular, I have examined the Ismagilov patents, including the asserted claims and the prosecution histories.

15. Based on my investigation and preparation in connection with this matter and my prior experience in the relevant field, I have developed an understanding of the teachings of the prior art. I have been asked to compare the asserted claims of the Ismagilov patents against the teachings of the prior art. The details of my analysis are provided below.

16. In my analysis of the validity of the '407 patent, I only examined the validity of the claims asserted in this suit: independent claim 1 and dependent claims 2-5, 8-11, and 13.

17. In my analysis of the validity of the '193 patent, I only examined the validity of the claims asserted in this suit: independent claim 1 and dependent claims 2-8, and 11.

18. In my analysis of the validity of the '148 patent, I only examined the validity of the claims asserted in this suit: independent claim 1 and dependent claims 2-3, and 6-8.

19. In my analysis of the validity of the '091 patent, I only examined the validity of the claims asserted in this suit: independent claims 1, 36, 37, and 57, and dependent claims 2-3, 5-6, 11, 27, 29, 31, 33, 35, 38-39, 43, 53, 56, and 58.

20. In my analysis of the validity of the '083 patent, I only examined the validity of the claims asserted in this suit: independent claims 1, 20, and 31, and dependent claims 2, 9-13, 21-22, and 26.

21. I am defining "asserted claims" as the claims listed in Plaintiffs' First Supplemental Response to 10X's Interrogatory No. 4. I reserve all rights to address any claims not listed in Plaintiffs' first supplemental response that Bio-Rad subsequently argues are

infringed.

22. It is my opinion that all asserted claims of the Ismagilov patents are invalid for at least the reasons set forth in detail below.

V. LEGAL STANDARDS

23. I have been informed of a number of legal standards that govern my analysis as set forth in this report. These legal standards have governed my analysis and opinions in this case.

A. Claim Construction

24. I understand that claim construction is a legal issue for the Court. I understand that in construing patent claims, courts seek to determine what a person of ordinary skill in the art would understand the claims to mean primarily in light of the intrinsic evidence of record, including the written description (e.g., specification), the drawings, and the prosecution history.

25. I have read and understand the Court's claim construction ruling, and have applied the Court's claim constructions throughout this report.

B. Invalidity Legal Standards

26. I understand that there are a number of legal factors or requirements that may be considered in determining whether the claims of a patent are valid or not. I also understand that, although the claims of an issued patent are presumed valid, those claims can be shown to be invalid by clear and convincing evidence that they fail to comply with one or more requirements of patentability. Notably, in situations where (as here) the Patent Office did not have all relevant information at its disposal during prosecution of the patents at issue, the considered judgment of the Patent Office in issuing those patents may lose significant force.

27. In forming the opinions expressed in this report, I have applied the following legal principles.

1. Prior Art

28. I understand that “prior art” includes what was patented or described in a printed publication in the United States or a foreign country or in public use or on sale in the United States more than one year before the filing date of the patent under analysis. I understand that “prior art” also includes what was known or used by others in the United States, or patented or described in a printed publication in the United States or a foreign country, before the date of invention for the patent under analysis. I understand that “prior art” also includes patents granted on applications for a patent by others filed in the United States before the date of invention for the patent under analysis. I understand that work of others is “prior art” if it was either (a) conceived and reduced to practice before the asserted date of invention, or (b) conceived before and diligently reduced to practice after the asserted date of conception.

2. Anticipation

29. I understand that a person is not entitled to a patent if the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent. *See* 35 U.S.C. § 102(a).

30. I understand that a person is not entitled to a patent if the invention was patented or described in a printed publication in this or a foreign country or was in public use or on sale in this country more than one year prior to the date of the application for patent in the United States. *See* 35 U.S.C. § 102(b). It is my further understanding that a sale or offer for sale may invalidate a patent under this section if what is sold or offered for sale is “ready for patenting,” i.e., it has either been reduced to practice or sufficient preparations have been made by the inventor to enable one of skill in the art to practice the invention. However, the parties to the transaction need not recognize that the product possesses the claimed characteristics.

31. I understand that a person is not entitled to a patent if the invention was described

in a published application for a patent filed by another in the United States before the invention by the patent applicant, or a patent granted on an application for patent by another filed in the United States before the invention by the patent applicant. *See* 35 U.S.C. § 102(e).

32. I understand that a person is not entitled to a patent if he or she did not invent the subject matter sought to be patented (sometimes known as “derivation”). *See* 35 U.S.C. § 102(f).

33. I understand that a person is not entitled to a patent if before such person’s invention thereof, the invention was made in this country by another inventor who had not abandoned, suppressed, or concealed it. *See* 35 U.S.C. § 102(g)(2). In determining priority of invention, one must consider the respective dates of conception and reduction to practice of the invention, and also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

34. Consistent with these principles, I understand that a patent claim is invalid for a lack of “novelty” (also called “anticipation”) if what is claimed is not new. Anticipation occurs if, within the “four corners” of a single prior art reference, each and every limitation of the patent claim is disclosed, either explicitly or inherently. I have been informed that a claim limitation may be inherently disclosed where it would have been necessarily present in the prior art device or method.

3. Obviousness

35. I understand that a patent claim may be found invalid as obvious if, at the time when the invention was made, the subject matter of the claim, considered as a whole, would have been obvious to a person having ordinary skill in the field of the technology (the “art”) to which the claimed subject matter belongs.

36. I understand that the following factors should be considered in analyzing obviousness: (1) the scope and content of the prior art; (2) the differences between the prior art

and the claims; and (3) the level of ordinary skill in the pertinent art. I also understand that certain other factors known as “secondary considerations” such as commercial success, unexpected results, long felt but unsolved need, industry acclaim, simultaneous invention, copying by others, skepticism by experts in the field, and failure of others may be utilized as indicia of nonobviousness. I understand, however, that secondary considerations should be connected, or have a “nexus,” with the invention claimed in the patent at issue.

37. I understand that a person of ordinary skill in the art is assumed to have knowledge of all prior art. I understand that one skilled in the art can combine various prior art references based on the teachings of those prior art references, the general knowledge present in the art, or common sense.

38. I understand that the principles relating to a “motivation,” “suggestion,” or “teaching” in the prior art to combine references to produce the claimed purported invention is one approach that may be taken in a validity analysis. I understand that the suggestion or motivation may be either explicit or implicit, may come from knowledge generally available to one of ordinary skill in the art, and may come from the nature of the problem to be solved. The test for an implicit motivation, suggestion, or teaching is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art. The problem examined is not the specific problem solved by the invention but the general problem that confronted the inventor before the invention was made. I understand that one should avoid “hindsight bias” and ex post reasoning in performing an obviousness analysis. But this does not mean that a person of ordinary skill in the art for purposes of the obviousness inquiry does not have recourse to common sense.

39. I understand that when determining whether a patent claim is obvious in light of the prior art, neither the particular motivation for the patent nor the stated purpose of the patentee is controlling. The primary inquiry has to do with the objective reach of the claims, and that if those claims extend to something that is obvious, then the entire patent claim is invalid.

40. I understand one way that a patent can be found obvious is if there existed at the time of the invention a known problem for which there was an obvious solution encompassed by the patent's claims. I understand that a motivation to combine various prior art references to solve a particular problem may come from a variety of sources, including market demand or scientific literature. I understand that a need or problem known in the field at the time of the invention can also provide a reason to combine prior art references and render a patent claim invalid for obviousness.

41. I understand that familiar items may have obvious uses beyond their primary purpose, and that a person of ordinary skill in the art will be able to fit the teachings of multiple prior art references together "like the pieces of a puzzle." I understand that a person of ordinary skill is also a person of at least ordinary creativity.

42. I understand when there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If these finite number of predictable solutions lead to the anticipated success, I understand that the invention is likely the product of ordinary skill and common sense, and not of any sort of innovation. I understand that the fact that a combination was obvious to try might also show that it was obvious, and hence invalid, under the patent laws.

43. I understand that if a patent claims a combination of familiar elements according

to known methods, the combination is likely to be obvious when it does no more than yield predictable results. Thus, if a person of ordinary skill in the art can implement a predictable variation, an invention is likely obvious. I understand that combining embodiments disclosed near each other in a prior art reference would not ordinarily require a leap of inventiveness.

4. *Written Description Requirement*

44. I understand that a patent must contain a written description of a claimed invention independent of the requirements to enable one skilled in the art at the time to make and use the invention. I understand that the implication of this requirement is that the full scope of the claims of a patent must be supported by the specification and applicable drawings. Furthermore, the written description in the specification must be such that one of ordinary skill in the art would understand from the disclosure that the patentee had actually invented what he or she now says is claimed and that the specification must convey with reasonable clarity to those of ordinary skill in the art at the time that, as of the filing date, the patentee was in possession of the invention.

5. *Enablement*

45. I understand that the specification must describe the claimed invention and the manner and process of making and using it in such terms as would enable any person skilled in the art to make and use the invention, without needing to engage in undue experimentation. I understand that the test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. I further understand that if the scope of a patent claim purports to cover a range, then the full scope of the claim must be enabled.

46. I understand that there is a lack of enablement where information is missing about one or more essential parts or relationships between parts which one skilled in the art could not develop without undue experimentation.

47. I understand that if a claim describes a device that cannot work, for example, because the claim describes something impossible to accomplish (such as perpetual motion), then the claimed invention is inoperable. I understand that an inoperable invention is not enabled. That is, it is not possible to enable a person of ordinary skill in the art to do something that cannot be done. I understand also that an inoperable invention is not considered “useful” under the patent law.

48. I understand that to show lack of enablement, it is not necessary to prove an intent by the patent applicant to withhold any particular information. Rather, I understand that all that is required is a failure to teach how to practice the invention claimed in the patent.

6. *Indefiniteness*

49. I understand that a patent must conclude with claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention. I understand that the scope of the claims must be sufficiently definite to inform the public of the bounds of the invention, *i.e.*, the subject matter covered by the patent. Thus, I understand that a claim must allow one skilled in the art to determine the bounds of the claim when read in light of the specification.

50. I understand that the focus of an inquiry into indefiniteness is whether the claims, read in light of the specification delineating the patent, and the prosecution history, fail to inform, with reasonable certainty, those skilled in the art at the time of the invention about the scope of the invention.

7. *Conception and Reduction to Practice*

51. I understand that conception is defined as the complete performance of the mental part of the inventive act and that conception is deemed to have occurred upon the formation in the mind of the inventor(s) of a definite and permanent idea of the complete and operative

invention as it is thereafter to be applied in practice. Conception has also been defined as a disclosure of an invention that enables one skilled in the art to reduce the invention to practice without exercise of the inventive faculty. To establish conception, a party must show that the inventor had possession of every feature recited in the claim and that every limitation of the claim must have been known to the inventor at the time of the alleged conception. Conception must be proved by corroborating evidence: evidence of the inventive facts must not rest alone on the testimony of the alleged inventor.

52. I understand that a reduction to practice can be actual or constructive. I understand that an invention is actually reduced to practice when the inventor has constructed an apparatus or performed a process that meets every limitation of the claimed invention, and has appreciated that this apparatus or process is operative for its intended purpose. I understand that an inventor constructively reduces an invention to practice by filing an application for patenting that satisfies the enablement requirement and includes sufficient written description to establish that the inventor has possession of the invention.

53. I understand that a patentee is entitled to claim the filing date of an earlier application if either (1) the later patent application is a continuation or continuation-in-part of the earlier application, or (2) the earlier application is a provisional application, and the later application is filed within 12 months of the provisional application, as long as in either case, the corresponding disclosure in the parent application supports the later-claimed subject matter.

8. *Level of Ordinary Skill in the Art*

54. It is my opinion that a person of ordinary skill in the art (a “POSA”) at the time of the alleged invention of the asserted claims would have (for example) knowledge of the scientific literature concerning microfluidic devices and the methods of using microfluidic devices and knowledge of the strategies for performing chemical and biological analysis is microfluidic

devices. Typically, a POSA at the time of the alleged invention would have a Ph.D. in chemistry, biochemistry, mechanical engineering, or a related discipline, with two years of experience in using, designing, or creating microfluidic devices. In determining the level of ordinary skill in the art, more education could compensate for less experience, and vice versa. For example, a POSA at the time of the alleged invention would have a M.S. or bachelor's degree in one of the disciplines listed with four or five years of additional relevant experience. Also, a POSA may have worked as part of a multidisciplinary team and drawn upon not only his or her own skills, but also taken advantage of certain specialized skills of others in the team.

VI. PRINCIPLES AND BACKGROUND KNOWLEDGE IN THE PRIOR ART

55. In the following section, I set forth a number of scientific principles relating to the subject matter of the Ismagilov patents that would have been known to those of ordinary skill in the art during the time prior to the purported invention of the Ismagilov patents. These principles are to be considered part of my detailed opinions regarding invalidity (as set forth in Sections IX through XIV below), and should be deemed fully incorporated by reference in that analysis.

A. Microfluidic Systems and Plugs

56. The Ismagilov patents relate to the field of microfluidics. “Microfluidics” describes the transport of fluids through sub-millimeter channels. The small channel dimensions and the flow format through these systems allow for smaller analyte¹ samples, faster processing time, larger numbers of different analytes, higher sensitivity of analysis, and easier imaging, among other desirable features.

57. Early research in microfluidics focused on technologies to “etch” or form these sub-millimeter channels in or on single chips (also referred to as “substrates”) composed of

¹ An analyte is a substance that is subject to analysis.

glass, polymer, silicon, metal, or other materials.

1. *Single Phase Systems*

58. Early microfluidic devices were often “single-phase” devices. In a single-phase device, a single fluid containing the sample is injected in each microchannel. The sample containing fluid is injected into the reservoirs at the end of the microchannels and the sample molecules within the fluid are separated by electric fields. Multiple fluids containing samples can be inserted into different reservoirs or sequentially into the same reservoir. The key obstacle to high-throughput assays in single-phase microfluidics is cross-contamination between separated molecules of the same fluid containing sample or between molecules from different fluids containing sample.

2. *Two Phase Systems*

59. Two-phase microfluidic devices utilize two immiscible fluids. Fluids are immiscible if they do not mix. One fluid, for example, an aqueous fluid, is segmented or encapsulated by a second immiscible fluid, for example an oil, in the form of “droplets”² or “slugs.” The first fluid is called the “dispersed phase” and the second fluid the “continuous phase” or “carrier fluid.” The development of two-phase devices began at least as early as the 1950’s, first in capillary systems and then in droplet emulsions before being adopted in microfluidic systems.

(a) Slug Systems

60. In 1957, Skeggs *et al.* described a capillary system for analyzing biological samples. Skeggs, L., et al., “Methods of and Apparatus for Analyzing Liquids Containing Crystalloid and Non-Crystalloid Constituents,” U.S. Patent No. 2,797,149 (filed on January 8,

² I will refer to “droplets” and “plugs” interchangeably throughout this report.

1953; issued June 25, 1957) (“Skeggs”) (10X-000003482-3496). In Skeggs, a biological sample was introduced into a main capillary channel. *Id.* at 4:29-34. With the use of valves, pockets of air were also injected into the channel in an alternating fashion with each biological sample, *id.* at 4:37-42, resulting in a segmented stream of fluids composed of alternating segments of air and biological sample, *id.* at 4:37-42. In the art, these segments of biological sample are referred to as “slugs.”

61. In 1962, Kessler, described a system that replaced air with an immiscible fluid, such as mineral oil, to create segmented streams of immiscible fluid and slugs of biological sample. Kessler, G., “Automatic Analysis with Fluid Segmentation,” U.S. Patent No. 3,047,367 (filed on December 1, 1959; issued July 31, 1962) (“Kessler”) (10X-000003497-3500) at 3:11-37. While these systems decreased sample cross-contamination as compared to single-phase systems, they had drawbacks. For example, contact between the channel wall and the slugs of biological sample allowed components of the sample to adhere to the channel wall and contaminate the following slugs of biological sample. Skeggs at 3:11-37; Kessler at 4:42-54.

62. To decrease this cross-contamination, POSAs began to construct microchannels using a material to which the carrier fluid had a higher wettability towards the channel than the aqueous fluid. Wettability measures the affinity between the liquid and the solid as a result of interaction between their respective molecules. Such affinity can be measured by an interaction energy or an adhesion force. Higher wettability results from stronger attractive adhesion force, also known as interfacial tension, between the carrier fluid and the channel surface. The higher adhesion force of the carrier fluid for the channel surface results in a film of carrier fluid between the aqueous fluid and the channel wall.

63. For example, in 1969 Smyth et al. described a device with channels made of

fluorinated hydrocarbon. Smythe, W., et al., “Method and Apparatus for Analysis,” U.S. Patent No. 3,479,141 (filed May 17, 1967; issued November 18, 1969) (“Smythe”) (10X-000003501-3505) at 2:35-61. Within the fluorinated hydrocarbon channel flows a silicone fluid which wets the channel walls and forms a film that adheres to the channel walls. *Id.* at 2:35-61 and Fig. 1. This film of silicone fluid further decreases cross-contamination by preventing the adherence of material from the sample fluid to the channel wall. *Id.* at 2:50-58. As described in Smythe: “The aqueous sample segments do not touch this inner wall of the conduit, but, . . . rather, travel within the film of silicone.” *Id.* at 2:53-58. And “[t]he aqueous sample segments do not adhere to the silicone film and thus do not leave a deposit of sample thereon, to contaminate successive sample segments.” *Id.*

(b) Droplet Systems

64. Two-phase systems where the aqueous phase is fully or substantially surrounded by the continuous phase (droplet systems) were subsequently developed. Droplets were generally formed using one of two primary approaches: (1) flow focusing; and (2) T-junction. However, other techniques were also known.

65. Flow focusing refers to a mechanism for generating droplets from a continuously flowing dispersed phase from a feed channel or a capillary. The flow that is being “focused” is the flow of the carrier fluid which is typically supplied from two side channels on both sides of the feed channel. There is also a constriction or orifice downstream such that the flow area for the carrier fluid is significantly reduced downstream. The carrier fluid is hence being “focused.” The focused carrier fluid produces a low pressure or a high shear rate in front or around the continuously flowing dispersed fluid at the capillary to deform its shape into a conic droplet or an elongated jet, from which isolated droplets of the dispersed fluid are emitted. The figure below reproduced from Gañán-Calvo, A., “Generation of Steady Liquid Microthreads and

Micron-Sized Monodisperse Sprays in Gas Streams,” *Physical Review Letters*, 80(2): 285-288

(1998) (“Gañán-Calvo I”) (RDTX00008417-20) depicts this method:

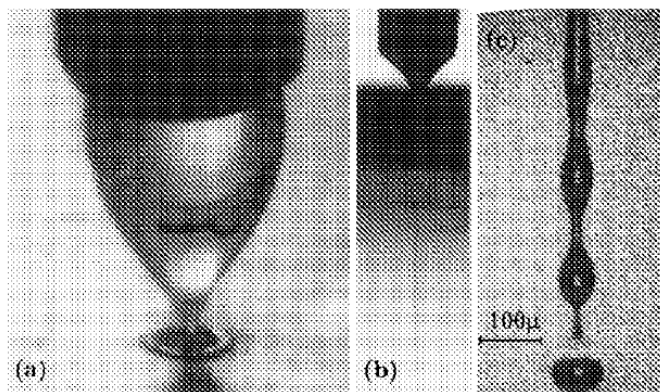


FIG. 1. (a) Typical view of the steady drop-jet mode. $R_o = 400 \mu\text{m}$, $D = 150 \mu\text{m}$, $H = 1 \text{ mm}$, $L = 300 \mu\text{m}$, $\Delta P_g = 20 \text{ kPa}$, $Q = 2 \mu\text{liter s}^{-1}$. Liquid: water. (b) General view of the cone-jet configuration with the steady liquid thread crossing the plate. $R_o = 200 \mu\text{m}$, $D = 150 \mu\text{m}$, $L = 350 \mu\text{m}$, $\Delta P_g = 5 \text{ kPa}$, $Q = 0.5 \mu\text{liter s}^{-1}$. Liquid: ethanol. (c) Close-up view of the breakup region taken with an ultrahigh speed video camera (shutter time 70 ns), showing a highly regular axisymmetric breakup pattern.

66. Unlike flow focusing, T-junction does not focus the carrier fluid to produce high pressure or high shear rate. Instead, it uses continuous carrier fluid flow in a main channel to shear off droplets of the dispersed fluid that is being fed continuously from one or multiple side channels. While there could be multiple side channels and the channels do not need to be perpendicular to the main channel, this method is generally called the “T-Junction” method. The figure below reproduced from Thorsen, T., et al., “Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device,” *Physical Review Letters*, 86(18): 4163-4166 (2001) (“Thorsen”) (RDTX00004647-4650) depicts this method:

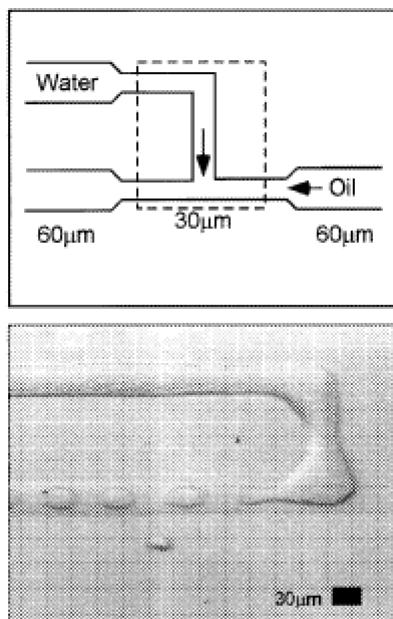


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

67. A substantial body of literature describing techniques for droplet generation, including flow focusing and T junction, existed prior to the purported invention of the Ismagilov patents.

68. At least as early as 1982, Shaw Stewart described “a method of combining chemical reagents” where the reagents are dispensed in “discrete volumes of droplets separated from each other by an inert immiscible liquid.” Shaw Stewart, P., “Combining chemical reagents,” UK Patent Application Publication GB 2,097,692 A (filed on January 11, 1982; published on November 10, 1982) (“Shaw Stewart”) (10X-000001053-1065) at (57). “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier flows down the tube. When each droplet almost spans the tube, it will be broken off.” *Id.* at 1:83-88. Figure 1 of Shaw Stewart, reproduced below, depicts this method:

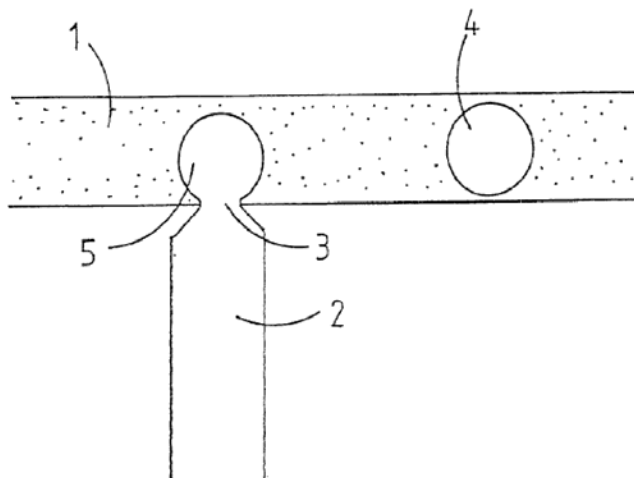


Figure 1.

69. In 1998, Gañán-Calvo described a flow-focusing water droplet generation technique that uses a highly focused gas flow into a small opening. Gañán-Calvo I. The low pressure of the accelerating gas flow at the opening pulls a liquid droplet at the end of a capillary into a cone. Gañán-Calvo I at 285. A small jet from the cone tip is pulled through the opening and the exiting jet then breaks up into water droplets. *Id.* In 2001, Gañán-Calvo and Gordillo reversed the phases and used flow focusing of an outer liquid to pull a gas filament through the opening. The gas filament pinches off into micron-sized gas bubbles upon exiting the opening. Gañán-Calvo, A. and Gordillo, J., “Perfectly Monodisperse Microbubbling by Capillary Flow Focusing,” *Physical Review*, 87:274501-1 - 274501-4 (2004) (“Gañán-Calvo II”) (10X0002555250-53).

70. In 2000, Umbanhowar produced droplets by pinching off droplets at a capillary tip using a flowing dispersed phase and flowing continuous phase. Umbanhowar, P.B., et al., “Monodisperse Emulsion Generation via Drop break Off in a Coflowing Stream,” *Langmuir*, 16: 347-351 (2000) (“Umbanhowar”) (RDTX00004496-4500). Umbanhowar described that “drops form at the tip of the [tapered] capillary and then detach when they reach a size where the

[viscous] drag force due to the coflowing [continuous] liquid exceeds the interfacial tension.” Ubanhowar at 347. Surfactants were added in the continuous oil phase to reduce the interfacial tension. *Id.*

71. As another example, in 2000, Sugiura et al. described a microfluidic device that generates droplets due to structure changes in the micro-channel. Sugiura, S., et al., “Preparation of Monodispersed Solid Lipid Microspheres Using a Microchannel Emulsification Technique,” *Journal of Colloid and Interface Science*, 227: 95-103 (2000) (“Sugiura I”) (10X-000255656-64). The dispersed liquid (oil) is driven into a series of small channels. Sugiura I at 96-97. The small channels feed into a perpendicular main channel with a sudden increase in the channel width at a “terrace” followed by a sudden increase in the channel height at a “well.” *Id.* This change in channel geometry results in droplets being pinched off into a flowing immiscible continuous phase (water) in the main channel. *Id.* Addition of surfactant was found to stabilize droplet generation. *Id.* at 98-100. In 1999, Kobayashi et al. used this device to form monodisperse oil-in-water emulsions. Kobayashi, I., et al., “Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels,” *Food Sci. Technol. Res.*, 5(4): 350-355 (1999) (“Kobayashi”) (10X-000250835-840). Kobayashi described adjusting various parameters of the system to achieve monodispersity among its droplets, including the pressures applied to the dispersed phase fluid, the concentrations of surfactant, among others. *Id.* at 350-51.

72. In 2001, Kawakatsu et al used this device to generate dispersed water droplets in a flowing continuous oil phase. Kawakatsu, T., et al., “The effect of the hydrophobicity of microchannels and components in water and oil phases on droplet formation in microchannel water-in-oil emulsification,” *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 179: 29-37

(2001) (“Kawakatsu”) (10X-000255355-63). That same year, Sugiura, S., et al. carried out polymerization reactions in dispersed oil droplets using this device. Sugiura, S., et al., “Synthesis of Polymeric microspheres with Narrow Size Distributions Employing Microchannel Emulsification,” *Macromol. Rapid Commun.*, 22: 773-778 (2001) (“Sugiura II”) (10X-000255665-70).

73. In 2001, J.R. Burns et al. described a “multiphase microreactor based upon the use of slug flow through a narrow channel.” Burns, J.R. and Ramshaw, C., “The intensification of rapid reactions in multiphase systems use slug flow in capillaries,” *Lab Chip*, 1:10-15 (2001) (“Burns (2001)”) (10X-000001710-1715) at Abstract. These “slugs” were formed by “the continuous flow of both [liquid] phases through T or cross-shaped intersections.” *Id.* at 11. Burns described that slugs were “generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting of the flow of the first phase into the channel and reversing the process.” *Id.* Figure 4 of Burns (2001) is reproduced below:

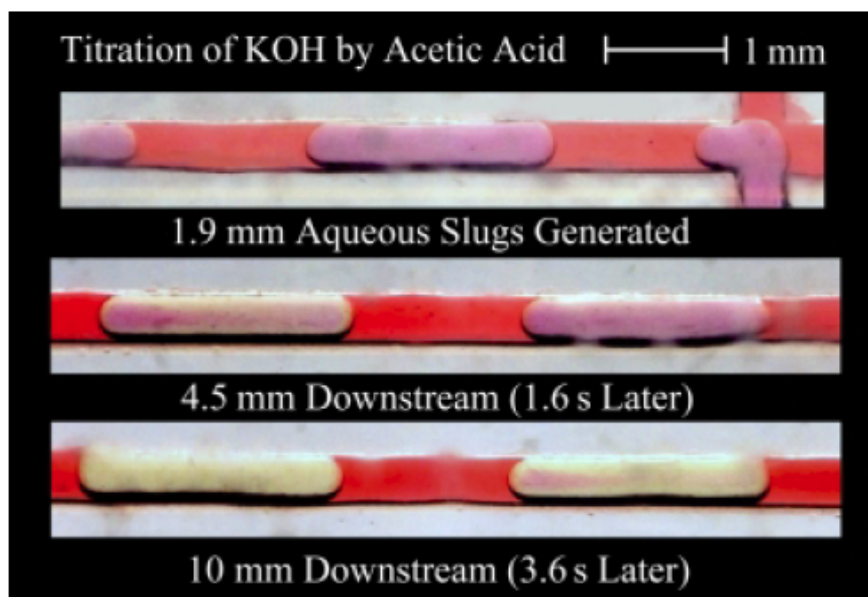


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

74. A simple acid-base reaction between acetic acid and NOH and acetic acid and KOH was conducted in these “slugs.” *Id.* at 11.³

75. Also in 2001, Thorsen et al. described the formation of droplets “by shearing one liquid into a second immiscible one, often in the presence of a surfactant.” Thorsen at 4163. Figure 1 of Thorsen is reproduced below.

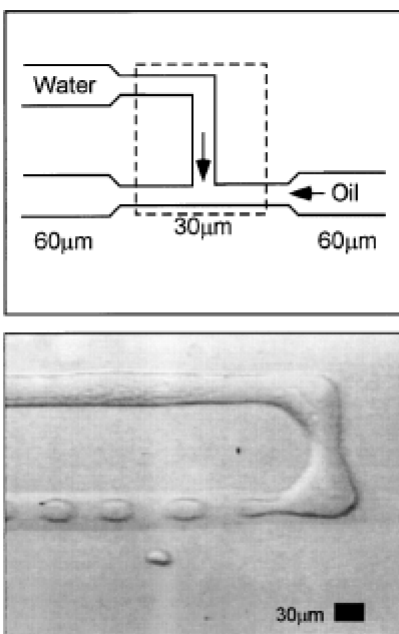
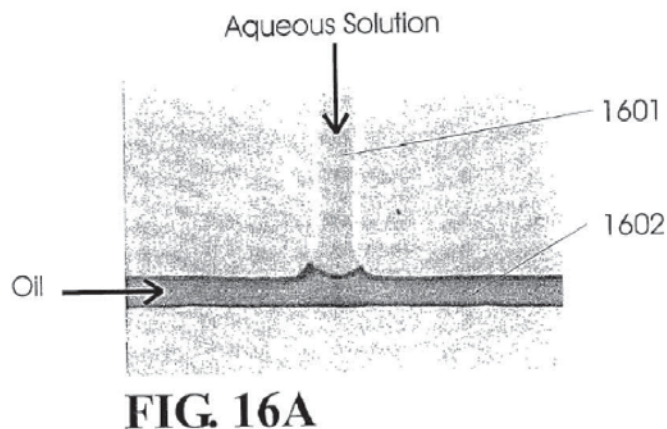


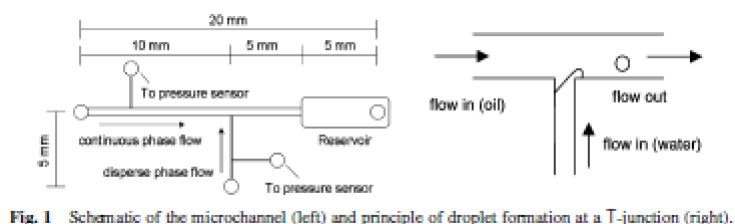
FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

76. Also in 2001, Quake et al. described “[a] microfluidic device for analyzing and/or sorting biological materials.” Quake, S. and Thorsen, T., “Microfabricated Crossflow Devices and Methods,” U.S. Patent Application Publication No. 2002/0058332 (filed September 4, 2001; published May 16, 2002) (“Quake”) (10X-000002812-2870), at Abstract. In Quake’s microfluidic system, droplets formed when a continuously flowing aqueous solution contacted a continuously flowing oil. *Id.*, ¶ [0003]. Figure 16A of Quake is reproduced below:

³ As discussed below, I disagree with the Burns (2001) characterization of “slugs.”



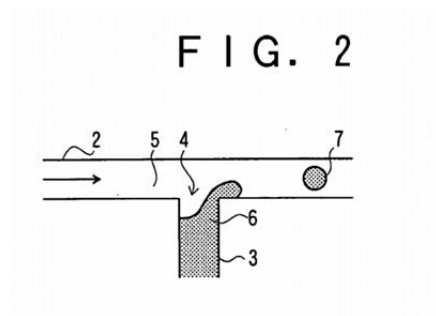
77. In 2002, Nisisako et al. described “droplet-based chemical reactors” generated “[w]ith oil as the continuous phase and water as the dispersed phase.” Nisisako, T., et al., “Droplet formatting in a microchannel network,” *Lab Chip*, 2:24-26 (2002) (“Nisisako”) (10X-000001800-1802) at 24. For example, Nisisako described that “pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *Id.* Figure 1 or Nisisako is reproduced below:



78. Nisisako envisioned the use of these droplets as chemical reactors. *Id.* at 26.

79. In early 2001 Higuchi et al. developed a microreactor in which a dispersed phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersed phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi, T., “Process for Producing Emulsion and Microcapsules and

Apparatus Therefor,” U.S. Application No. 2004/0068019 (filed February 13, 2002; published April 8, 2004) (“Higuchi I”) (10X-000255262-80). Figure 2 of Higuchi I, reproduced below, depicts this microreactor.



80. This microreactor was specifically intended to be used to perform chemical reactions. Taniguchi et al., “Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media,” *Royal Society of Chemistry* (2002) (“Taniguchi”) (RDTX00000636-40).

81. In 2002, Todd Thorsen developed a droplet reactor where “[c]ells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction. (Figure 4.1).” Thorsen, T., “Microfluidic Technologies for High-Throughput Screening Applications,” Doctoral Thesis, California Institute of Technology (2002) (<http://thesis.library.caltech.edu/4701/>) (“Thorsen Thesis”) (10X-000255685-866) at 94-108. This droplet reactor is depicted in Figure 2.1 of the Thorsen Thesis, reproduced below:

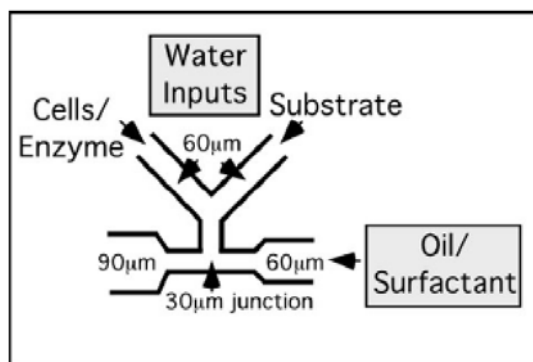


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

B. Microfluidic Systems and Conducting Reactions

82. In conjunction with the development of droplet microfluidic systems, researchers generally explored the use of microfluidic systems as chemical reactors and developed a substantial body of literature of this topic.

83. In 1998, Tawfik et al. explained that biochemical reactions remain active in pico-liter (2.6 microns diameter) dispersed water droplets in oil. Tawfik, D. and Griffiths, A., “Man-made cell-like compartments for molecular evolution,” *Nature Biotechnology*, 16: 652-656 (1998) (“Tawfik”) (10X-000005222-5228). “In the aqueous compartment of these emulsions, genes could be transcribed and translated to yield active proteins with the efficiency of non-emulsified reactions.” *Id.* at 652. The water droplets were stabilized with surfactants. *Id.*

84. In 2000, Wang described “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang, L., et al. “Method and Apparatus for Production of Small Particles of Micrometer or Nanometer Size,” Int. Pub. No. WO 00/23181 (filed October 19, 1998; published April 27, 2000) (“Wang”) (10X-000001066-1080), at Abstract. The reagent solution and precipitating solution could be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. *Id.* at 2:19-26. These small particles were “micrometer or nanometer size.” *Id.* at 1:4-5.

85. In 2001, Ghadessy et al. “described a compartmentalized self-replication, a strategy for the directed evolution of enzymes, especially polymerases” in pico-liter dispersed water emulsions in oil. Ghadessy, F., et al., “Directed evolution of polymerase function by compartmentalized self-replication,” *Pro. Natl. Acad. Sci.*, 98: 4552-4557 (2001) (“Ghadessy”) (RDTX00008015-8020).

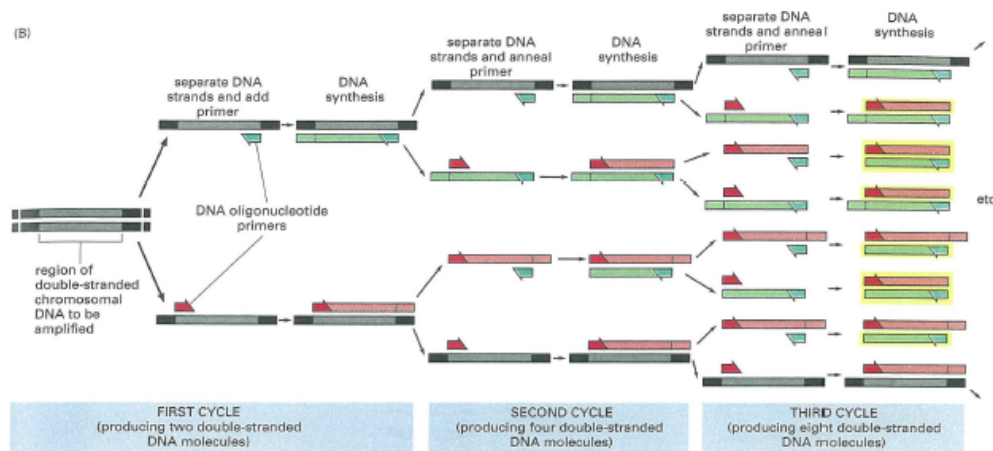
86. In 2002, Seki described a “droplet mechanism” for the formation of droplets for

use “where an analysis or a chemical reaction is conducted.” Seki, M., et al., “Control Mechanism for Trace Quantity of Liquid,” U.S. Patent Application Publication 2002/0195463 A1 (filed on May 30, 2001; published on December 26, 2002) (“Seki”) (10X-000251977-2006) at [0002]. The control mechanism “comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel . . . whereby the third flow channel links the first flow channel to the second flow channel.” *Id.* at [0018]. Droplets were formed when “a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” *Id.* These droplets can be merged to perform chemical reactions. *See, e.g., id.* at [0139]. As the microchip was transparent, these reactions could be observed. *Id.* at [0143].

87. One application that was particularly appealing to scientists in the field was the use of small volumes to conduct PCR (polymerase chain reaction).

88. PCR is a polymerization reaction that is used to amplify regions of DNA. DNA is a nucleic acid molecule composed of individual building blocks called “nucleotides.” Each nucleotide is associated with one of four bases: adenine (A), cytosine (C), guanine (G), and thymine (T). A DNA molecule consists of two strands coiled around each other in the shape of a double helix. Because of their complementary chemistry, each nucleotide base binds specifically to a particular nucleotide base on the opposite strand: A to T, and C to G. PCR relies on complementary base pairing and biochemical reactions to make copies of DNA molecules using existing DNA as a template. As depicted in the figure below, the reaction comprises three steps

(a cycle). First, the strands of the DNA are separated. *Alberts, B., et al., Molecular Biology Of The Cell* (4 ed. 2002) (“Alberts”) (10X-000254879-960) at 509. Second, two synthetic DNA oligonucleotide primers bind to the DNA strands. *Id.* Third, the DNA strands are incubated with an enzyme called DNA polymerase and free nucleotides. Starting from the primers, DNA polymerase synthesizes a new strand complementary to the existing strand using the free nucleotides introduced into the reaction. *Id.* The cycle is performed over and over to amplify the sequence bracketed by the primers. *Id.*



Id.

89. The number of cycles required for most applications range from 10 to 40. The duration of each cycle depends on the thermal capacitance of the reactor, which is proportional to the volume. Accordingly, one advantage of a smaller PCR reactor is that the PCR amplification cycles can be completed more rapidly.⁴ As Bio-Rad’s expert Dr. Shelley Anna observed in another proceeding, by the end of 2000, “it was well known in the field of microfluidics to

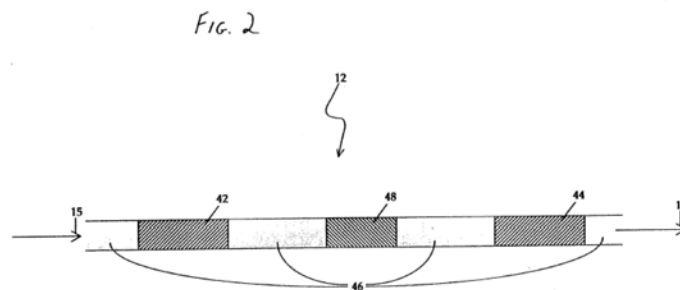
⁴ In another proceeding, Bio-Rad’s expert Dr. Shelley Anna confirmed this point, noting that “isolating smaller volumes of material (e.g., smaller droplets) was known [by mid-2000] to be particularly helpful for increasing the speed and efficiency of certain analytical tasks and procedures,” and “reducing channel sizes, using known manufacturing techniques” was “a well-known way of achieving higher speeds and efficiencies” of reactions occurring in microfluidic devices. IPR2015-00009 Ex. 1002 ¶ 63.

perform nucleotide amplification using microfabricated devices,” and PCR was “a well-known method for amplification of nucleic acids.” Declaration of Professor Shelley Anna, Ph.D. (dated October 1, 2014), IPR2015-0010, ¶¶ 105, 102.

90. A small volume PCR reactor also allows for higher sensitivity than a larger reactor. For example, a single nucleic acid can be amplified and detected in a single droplet. Unlike large PCR reactors, large number of droplets can be generated so that the number of a particular nucleic acid can be accurately quantified over a large range. This capability has multiple applications. For example, it allows for accurate quantification of pathogen nucleic acids and circulating tumor nucleic acids.

91. In light of these advantages, among others, using two phase microfluidic systems to segment or encapsulate reagents for PCR quickly gained traction.

92. In 1991, for example, Corbett et al. described a device for PCR amplification that comprised of “injecting the reaction mixture into a stream of carrier fluid, the reaction mixture being immiscible in the carrier fluid.” Corbett, J., et al., “Device and Method for the Automated Cycling of Solutions Between Two or More Temperatures,” U.S. Patent No. 5,270,183 (filed on February 8, 1991; issued on December 14, 1993)) (“Corbett”) (10X-000003512-3521) at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Figure 2 of Corbett is reproduced below:



93. Corbett disclosed that the carrier fluid could be “either a silicon oil or mineral oil.” *Id.* at 7:37-39. Corbett further disclosed that the reaction mixture was 20 μL or less. *Id.* at 9:35-40.

94. In 1996, Mark A. Burns et al. described performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns, M., et al., “Microfabricated structures for integrated DNA analysis,” *Proc. Natl. Acad. Sci. USA*, 93: 5556-5561 (1996) (“Burns (1996)”) (10X-000001037-1042) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C).” *Id.* Figure 1 of Burns (1996) is reproduced below:

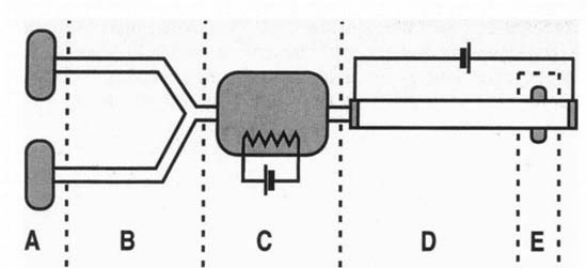


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

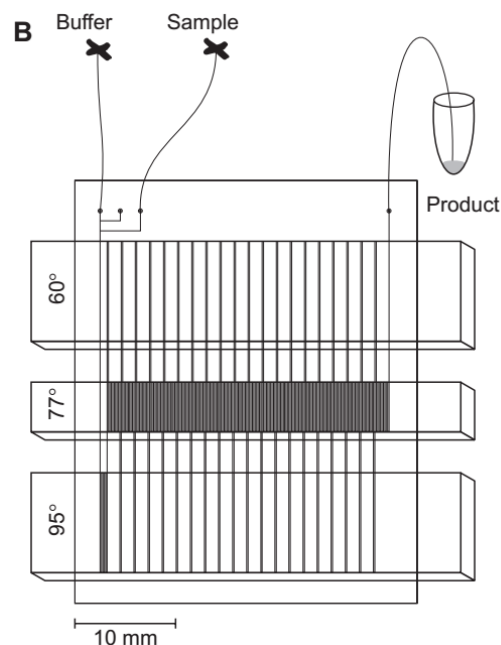
95. Also in 1996, Brody et al. explained that “[t]here has been a surge of interest in the ‘lab-on-a-chip’ concept, which involves the miniaturization of many chemical processes onto a single silicon chip.” Brody, J., et al., “Biotechnology at Low Reynolds Numbers,” *Biophysical Journal*, 71:3430-3441 (1996) (“Brody”) (10X-000001698-1709) at 3430.

Because these systems allow one to manipulate single cells, and even single macromolecules, there is great interest in the biotechnology community in using microfluid systems for analytical tests. For example, in the polymerase chain

reaction, amplification of DNA in a microenvironment is attractive both because the temperature can be rapidly cycled and because the sample volume is extremely small.

Id. at 3430.

96. By 1998, Kopp et al. had successfully implemented continuous flow PCR on a chip. Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Kopp, M., et al., “Chemical Amplification: Continuous-Flow PCR on a Chip,” *Science*, 280: 1046-1048 (1998) (“Kopp”) (RDTX00006580-6582) at 1046. Building on that body of work, Kopp et al. developed a “chemical amplifier [that] was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” *Id.* at 1046. In Kopp’s device, the sample is moved through temperature zones on a glass microchip. *Id.* Figure 1B of Kopp is reproduced below:



97. In 1999, Vogelstein et al. reported various applications for single-molecule microfluidic PCR. Vogelstein, B. and Kinzler, K., “Digital PCR,” *Proc. Natl. Acad. Sci.*, 96:

9236–9241 (1999) (“Vogelstein”) (RDTX00003738-3743) at 9239-41. Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.*

98. In 2000, Ferrance et al. provided an overview of known techniques and apparatus for performing continuous flow PCR in microfabricated chips. Ferrance, J., et al., “Toward effective PCR-based amplification of DNA on microfabricated chips,” *Capillary Electrophoresis of Nucleic Acids*, 2: 191-204, (2001) (“Ferrance”) (10X-000255217-30). Ferrance et al. explained:

To utilize this sequence most efficiently there should be a continuous flow from sample collection to diagnosis. . . .

The same advantages of reduced time, sample, and reagents brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions. Both capillaries and microchip devices have reduced the time needed for PCR but cycle times concomitant with the fast separations now possible are still being developed . . .

Although capillaries have proven useful for small scale PCR, true integration of the PCR and fast separation steps will require a microchip device where continuous flow of the PCR products to the separation channels is achievable on a single coordinated platform (see Note 2). With this type of platform, integration

not only of the last two steps shown in Fig. 1 is possible, but total integration of the complete process. . . .

Ferrance at 191-192.

99. In 2001, Lagally described an integrated microfluidic device for “the stochastic PCR amplification and analysis of single-molecule DNA templates.” Lagally, E.T., et al., “Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device,” *Anal. Chem.* 73:565-570 (2001) (“Lagally”) (10X-000002375-2380), at Abstract. In this system, “[t]he PCR chambers are connected to a common sample bus through a set of valves” and “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at 565. Figure 1 of Lagally is reproduced below:

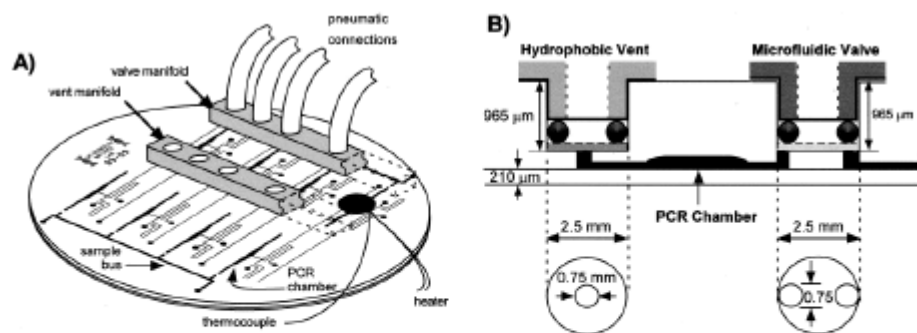


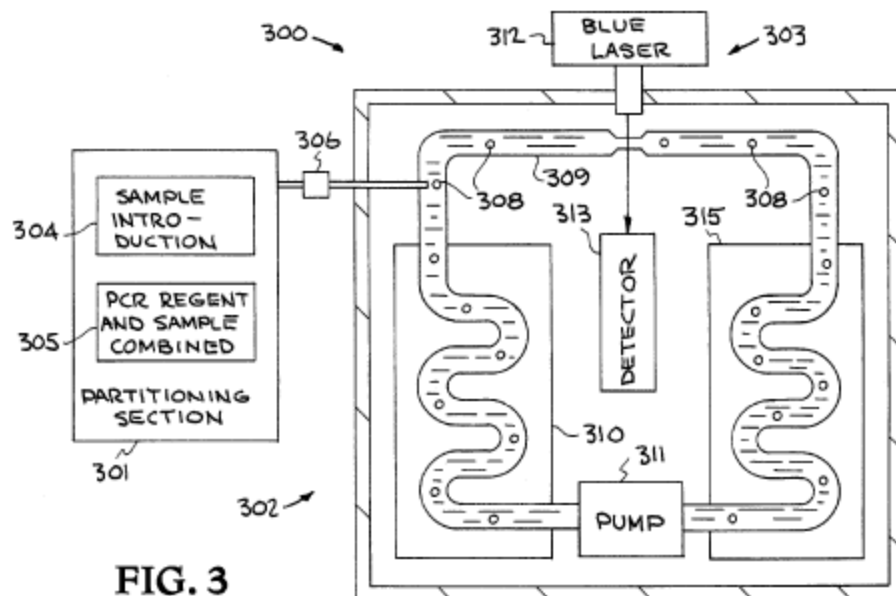
Figure 1. (A) Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber. (B) Detailed schematic of microfluidic valves and hydrophobic vents. Sample is loaded from the right by opening the valve using vacuum (30 mmHg) and forcing the sample under the membrane using pressure (10–12 psi); vacuum is simultaneously applied at the vent to evacuate the air from the chamber. The sample stops at the vent, and the valve is pressure-sealed to enclose the sample. Dead volumes for the valves and vents are ~50 nL.

100. Also in 2001, Chiou, J., et al. described “[a] novel thermocycling machine based on a microcapillary equipped with bidirectional pressure-driven flow and in situ optical position sensors.” Chiou, J., et al., “A Closed-Cycle Capillary Polymerase Chain Reaction Machine,” *Anal. Chem.*, 73: 2018-2021 (2001) (“Chiou”) (RDTX00008767-8770) at 2018. “A 1-μL droplet of reaction mixture moves between three heat zones in a 1-mm-i.d., oil-filled capillary” *Id.*

101. In 2002, Curcio described a chip-based PCR device where “aqueous reaction

mixtures are separated from each other by an immiscible organic fluid” and “transported in segmented flow through the reactor.” Curcio, M., “Improved Techniques for High-Throughput Molecular Diagnostics,” Ph.D. Thesis, Department of Chemistry Division of Analytical Chemistry, Royal Institute of Technology SE-100 44 Stockholm Sweden (2002) (“Curcio”) (10X-000001914-2045) at 34. Both “sample and reagents are loaded” in the aqueous reaction mixtures. Curcio, Paper V at 1. Curcio utilized “[a] continuous segmented-flow method for sequential DNA amplification” using a “15 meter long narrow-bore Teflon tube, coiled such as to be repeatedly exposed to three different temperature zones, optimized according to a PCR protocol.” Curcio at 1. For the immiscible organic fluid, “[p]erfluorocarbons are particularly suitable for this application because of the superior hydrophobicity over other liquids and an extremely poor chemical affinity for biomolecules.” *Id.* Wall interaction was eliminated “where a layer of the organic phase is created between the channel surface and the droplet.” *Id.* at 36.

102. In 2003, Anderson et al. described “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson, B., et al., “Chemical Amplification Based on Fluid Partitioning,” U.S. Patent No. 7,041,481 B2 (filed March 14, 2003; issued May 9, 2006) (“Anderson”) (10X-000004019-4027) at Abstract. Figure 3 of Anderson is reproduced below:



C. Carrier Fluid

103. As these microfluidic systems developed in which droplets or microemulsions were used as reaction chambers, researchers in the field recognized the need for carrier fluids that would make it possible to properly stabilize and encapsulate microfluidic droplets. Accordingly, scientists started using surfactants to help stabilize the microfluidic droplets.

104. Surfactants, sometimes referred to as a “surface active agents,” typically have the basic structure depicted below:



Taylor, C., Chapter 8: Fluorinated surfactants in practice, in “Design and Selection of Performance Surfactants,” Karsa D.R. (ed.), Sheffield Academic Press (1999) (“Taylor”) (10X-

000005229-5276) at 272. The oval represents a “head” group and the straight line represents a “tail” group. *Id.* at 272. Surfactants are surface active because they concentrate at interfacial regions such as oil-water or liquid-solid interfaces. West, C., et al., “Surfactants and Subsurface Remediation,” *Envtl. Sci. Tech.*, 26: 2324 (1992) (“West”) (10X-000002406-2412) at 3. For example, in the microfluidic droplet systems described above where the droplet is aqueous and the carrier fluid is oil, a surfactant has a polar or ionic hydrophilic head group (which is attracted to water) and a nonpolar hydrophobic tail group (which is attracted to oil). *Id.*

105. In 1991, Delpuech et al. described “water droplets enclosed in a fine surfactant membrane and contained in a continuous oil/surfactant phase.” Delpuech, J.-J., et al., “Visco-Elastic, Isotropic Materials Based on Water, Fluorinate Sufactants and Fluorinated Oils, Process for Their Preparation, and Their Use in Various Fields, Such as Optics, Pharmacology and Electrodynamics,” U.S. Patent No. 5,185,099 (filed on November 14, 1991; issued February 9, 1993) (“Delpuech”) (10X-000003506-3511), at Abstract. This “compartmentalized structure can make it possible to achieve chemical reactions in a confined medium.” *Id.* at 5:3-5.

106. In 1994, Schubert et al. described the use of fluorinated oils and surfactants, which had the added benefit of chemical compatibility with biological materials. Schubert, K.V. and Kaler, E.W., “Microemulsifying fluorinated oils with mixtures of fluorinated and hydrogenated surfactants, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 84: 97-106 (1994) (“Schubert”) (10X-000002067–2076). For example, Schubert described “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” *Id.* at 97. Schubert noted that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

107. Others in the field also described emulsions using fluorinated surfactants. For

example, Krafft et al. described emulsions comprising a fluorocarbon hydrophobic phase. Krafft, M.P., et al., “Multiple Emulsions Comprising a Hydrophobic Continuous Phase,” U.S. Patent No. 5,980,936 (filed August 7, 1997; issued November 9, 1999) (“Krafft”) (10X-000251172-85), at Abstract. Exemplary fluorocarbons were provided. *Id.* at 9:24-51. Krafft also described that a “dispersing agent” or surfactant may also be used, and exemplary fluorinated surfactants were provided. *Id.* at 11:5-23. Like Schubert, Krafft noted the compatibility of fluorinated surfactants with biological molecules, stating that these “fluorochemical emulsions” were used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

108. In fact, the biocompatibility of fluorocarbons, such as fluorinated surfactants, was widely noted in the field prior to the Ismagilov patents. For example, in 1999, Ramsey et al. described “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nano- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, J.M., et al., “Microfluidic Devices for the Controlled Manipulation of Small Volumes,” U.S. Patent No. 6,524,456 B1 (filed on September 29, 1999; issued February 25, 2003) (“Ramsey”) (10X-000003848-64), at Abstract. The volumes of reaction fluid in Ramsey were separated by volumes of segmenting fluid which was preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Ramsey disclosed that suitable segmenting fluids included: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

109. Parris also disclosed the potential use of fluorocarbons in these types of systems, noting the “inert” quality of fluorocarbons. Parris, N., “Method for Analysis,” U.S. Patent No. 5,739,036 (Filed on August 15, 1997; issued April 14, 1998)) (“Parris”) (10X-000003543-3547).

Parris described an “analyzer apparatus for analyzing components of a sample.” *Id.* at Abstract. “The sample is passed through the conduit using a carrier liquid that is immiscible with the sample” *Id.* “Preferably, the carrier liquid is a fluorocarbon” *Id.* at 2:33-35. The “liquid samples are introduced in sequence into the conduit and separated by liquid fluorocarbons so that they don’t improperly mix.” *Id.* at 3:16-20. “[F]luorocarbons are selected to be relatively inert and typically have a water solubility of less than 10 ppm.” *Id.* at 3:20-24.

D. Two or More Aqueous Fluids

110. As the field of microfluidics developed, researchers also began exploring the use of two aqueous fluids both containing reagents, that could be combined so that a reaction could occur. As early as 1997, Kopf-Sill et al. described a microfluidic device with channels of varied depth which allowed for improved mixing. Kopf-Sill, A., et al., “Microfluidic Systems Incorporating Varied Channel Dimensions,” U.S. Patent No. 5,842,787 (filed on October 9, 1997; issued December 1, 1998) (“Kopf-Sill”) (10X-000003548-3564), at Abstract. The materials to be mixed were introduced in parallel through two channels that intersect a main channel. *Id.* at 14:3-16.

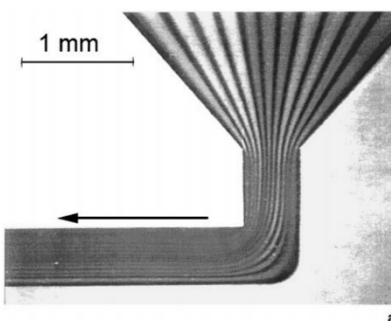
111. In 1999, Kenis et al. described a microfluidic system which involved introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis, P., et al., “Microfabrication Inside Capillaries Using Multiphase Laminar Flow Patterning,” *Science*, 285: 83-85 (1999) (“Kenis”) (10X-000002077-80) at 83. The liquid streams were “reactive species” and thus, a reaction could occur at the interface of the laminar streams. *Id.*

112. Also in 1999, Weigl et al. described a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl, B.H. and Yager, P., “Microfluidic Diffusion-Based Separation and Detection,” *Science*, 283: 346-347 (1999) (“Weigl”) (10X-000002081-84) at 346.

“The fluids interact during parallel flow until they exit the microstructure.” *Id.*

113. In 2000, Kenis et al. reported that a continuous flow microchannel reactor could be used for various fabrication reactions. Kenis, P., et al., “Fabrication inside Microchannels Using Fluid Flow,” *Accounts of Chemical Research*, American Chemical Society, 33(11) (2000) (“Whitesides”) (10X-000001852-1858). Kenis et al. explained that a wide range of technologies have already been used to conduct chemical reactions in microchannels, *id.* at 842, and described that the laminar flow which predominates in microchannel devices makes them well suited as microreactors because “laminar flow can be used to deliver reagents spatially inside capillaries with remarkable precision.” *Id.* at 841.

114. In 2001, Erbacher et al. reported on their development of a continuous flow microchannel reactor in which complete mixing of the reactants occurred within just a few seconds. Erbacher, C., et al., “Towards Integrated Continuous-Flow Chemical Reactors,” *Mikrochim. Acta*, 131: 19-24 (1999) (“Erbacher”) (10X-000251070-251075) at 19. Figure 4a of Erbacher is reproduced below:



E. Bio-Rad’s View of the State of the Art

115. I understand that in proceedings before the U.S. Patent Trial and Appeal Board, Bio-Rad made various statements about the state of microfluidic technology as of late 2000. *See generally* IPR2015-00009 Petition for Inter Partes Review, *Bio-Rad Laboratories, Inc. v. California Institute of Technology*, Patent No. 7,294,503 (dated October 1, 2014), (“IPR2015-

0009 Petition”); *see also id.* at 6; *see generally* IPR2015-00010 Petition for Inter Partes Review, *Bio-Rad Laboratories, Inc. v. California Institute of Technology*, Patent No. 8,252,539 (dated October 1, 2014), (“IPR2015-00010 Petition”); *see also id.* at 1. I understand that Bio-Rad succeeded in persuading the Patent Trial and Appeal Board that various claims in patents claiming priority to Quake were unpatentable over prior art. The prior art relied on included (1) Shaw Stewart, P., “Chemical Droplet Reactor,” Int. Pub. No. WO 84/02000 (filed on November 9, 1982; issued May 24, 1984) (“Shaw Stewart II”) (RDTX00012790-12810), which is related to the “Shaw Stewart” reference discussed throughout this report, (2) Burns, M., et al., “Microfabricated Isothermal Nucleic Acid Amplification Devices and Methods,” Int. Pub. No. WO 98/22625 (filed on November 19, 1997; issued May 28, 1998) (“Burns WO”) (10X-000250376-527), which describes the device described in the “Burns (1996)” reference discussed throughout this report, and (3) Kobayashi, I., et al., “Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels,” *Food Sci. Technol. Res.*, 5(4): 350-355 (1999) (“Kobayashi”) (10X-000250835-840), which is discussed in this report.

116. Bio-Rad’s statements before the Patent Trial and Appeal Board demonstrate its agreement that many basic aspects of droplet generation were well understood in the field by late 2000. Bio-Rad stated, for example, that by this time “achieving uniform droplets with regular periodicity” in a microfluidic device was “a well known optimization for chemical and biological reactions,” for example because it was “very well known that it [was] preferable to use repeatable, consistent volumes of reactants for chemical and biological reactions,” IPR2015-00009, Petition at 27, and “isolating smaller volumes of material (e.g., smaller droplets) was known [by mid-2000] to be particularly helpful for increasing the speed and efficiency of certain analytical tasks and procedures.” Declaration of Professor Shelley Anna, Ph.D. (dated October 1,

2014) (“IPR2015-00009 Ex. 1002”), ¶ 63. Similarly, Bio-Rad stated that the generation of “monodisperse oil-in-water emulsions [in] microchannels” by adjusting parameters including “pressures, concentrations of surfactant, etc.” was a “routine task that did not require undue experimentation” as of this time—as evidenced, for example, by the teachings of Kobayashi. *Id.* at 37. I agree with these statements regarding the knowledge of a POSA as of late 2000. In particular, all of this knowledge would have been equally available to a POSA as of the priority dates of the Ismagilov patents.

117. In the same proceedings, Bio-Rad also stated that the generation of droplets with particular desired properties (in particular, droplets useful for conducting chemical reactions) was well known by late 2000. Bio-Rad stated, for example, that in light of disclosures such as Shaw Stewart II and Burns WO, it was obvious by this time to “generat[e] droplets that also contain particles” by “includ[ing] particles in sample fluid prior to droplet formation,” *id.* at 22-23, that it was “a well known desire in the field of microfluidics . . . to isolate single particles and/or molecules for reactions and analysis,” *id.* at 30-31, and that modifying the “concentrations and/or amounts of reactants . . . in order to optimize reactions,” for example by isolating single particles in droplets, was “routine in microfluidics” as of that time. *Id.* at 31.

118. Bio-Rad further stated that a POSA as of late 2000 would have been able to use a microfluidic device as taught by Shaw Stewart II in view of Burns WO to create “droplets containing viral particles,” and that such use would be an “an obvious use of [that] device” because viral particles were “nothing more than a selection of a well known type of biological material” that were “routinely subject to analysis . . . in microfluidic devices” as of that time. *Id.* at 31. I agree with these statements regarding the knowledge of a POSA as of late 2000. In particular, all of this knowledge would have been equally available to a POSA as of the priority

dates of the Ismagilov patents.

119. Bio-Rad has also stated that “it would be immediately apparent to one of ordinary skill in the art to use biological samples such as nucleic acids . . . in the droplet formation process of [Shaw Stewart II] in order to perform reactions and analysis with such materials,” IPR2015-00010 Petition at 33, and that “performing nucleotide amplification reactions . . . was well known in the field of microfluidics prior to” late 2000. *Id.* at 35. I also agree with these statements. In particular, all of this knowledge would have been equally available to a POSA as of the priority dates of the Ismagilov patents.

120. I note that these statements contradict prior statements by The University of Chicago to the Patent Trial and Appeals Board that “as late as 2006, researchers characterized microfluidics as a field in ‘infancy.’” *See, e.g.*, The University of Chicago’s Preliminary Response to Petition for Inter Partes Review of U.S. Patent No. 8,889,083 Pursuant to 37 CFR § 42.107 (“IPR2015-01157 POPR”) at 18. Like Bio-Rad, I disagree with the University of Chicago’s statements.

VII. THE ASSERTED PATENTS

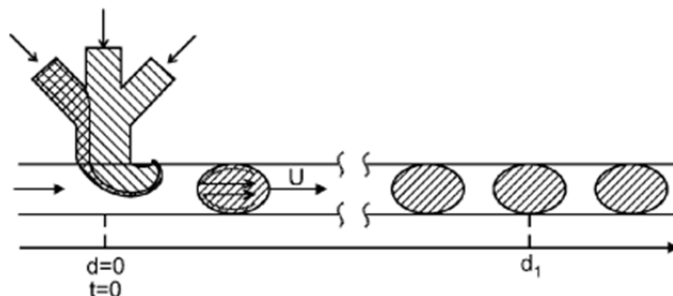
A. Background

1. ’407 Patent

121. The ’407 patent is directed generally to “microfabricated substrates and methods of conducting reactions within these substrates.” ’407 patent, at Abstract. The term “substrate,” as used throughout the Ismagilov patents, “refers to a layer or piece of material from which devices or chips are prepared or manufactured . . . [and] includes any substrate fabricated using any traditional or known microfabrication techniques.” ’407 patent at 11:57-61. Simply, a “substrate” is a microfluidic chip.

122. Generally, the ’407 patent claims methods of performing a reaction between a

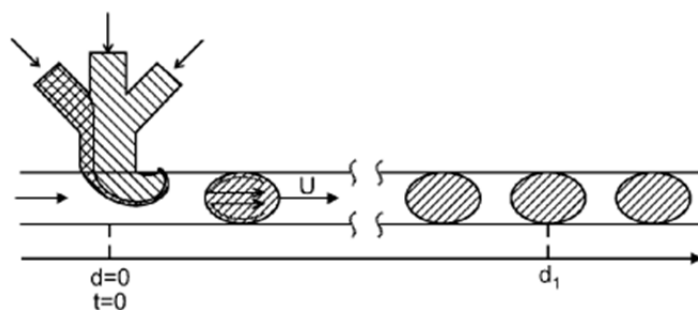
biological molecule and at least one reagent in a microfluidic system in plugs. The microfluidic system comprises at least two channels that have at least one junction. Plugs are formed by partitioning the flowing aqueous fluid with the flowing carrier fluid at the junction of the two channels into volumes of aqueous fluid substantially surrounded by the carrier fluid. Figure 2A of the '407 patent illustrates this process:



2. '193 Patent

123. The '193 patent is directed generally to “microfabricated substrates and methods of conducting reactions within these substrates.” '193 patent, at Abstract.

124. Generally, the '193 patent claims methods of performing autocatalytic reactions in plugs. The microfluidic system comprises at least two channels that have at least one junction. Plugs are formed by partitioning the flowing aqueous fluid with the flowing oil at the junction of the two channels into volumes of aqueous fluid substantially surrounded by the oil. Figure 2A of the '193 patent illustrates this process:



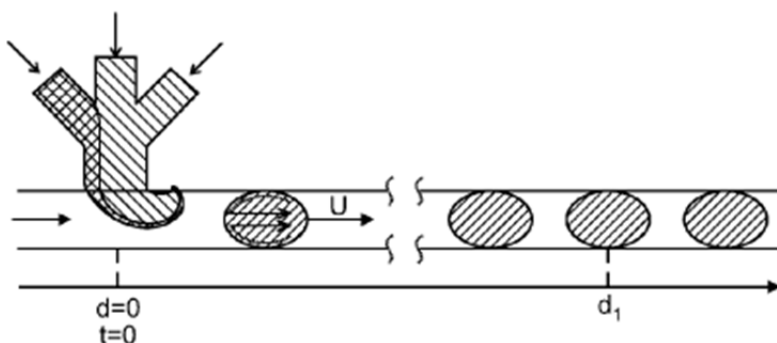
125. The claims further require that at least one plug comprises at least one substrate

molecule and reagents for conducting the autocatalytic reaction with the substrate molecule and providing conditions suitable for the autocatalytic reaction such that the substrate molecule is amplified in the at least one plug.

3. '148 Patent

126. The '148 patent is directed generally to “microfabricated substrates and methods of conducting reactions within these substrates.” '148 patent at Abstract.

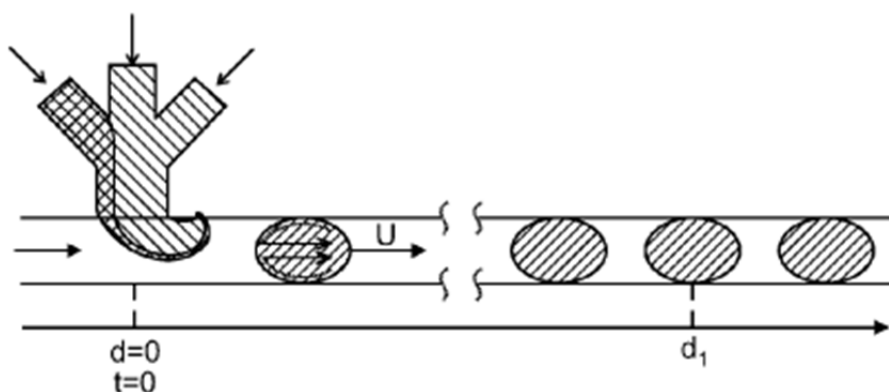
127. Generally, the '148 patent claims methods of amplifying a DNA or RNA target molecule by providing conditions suitable for PCR in plugs. The claims further require that the plugs have a uniform size of about 200 μm or less, that at least one plug comprises a single RNA or DNA target molecule and PCR reagents, and that the distribution of target DNA or RNA molecules in the plurality of plugs follows a Poisson distribution. The microfluidic system comprises at least one channel. Plugs are formed by controlling flow rates so that the continuously flowing carrier fluid partitions the continuously flowing aqueous fluid. Figure 2A of the '148 patent illustrates this process:



4. '091 Patent

128. The '091 patent is directed generally to “microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid.” '091 patent, at Abstract.

129. Generally, the '091 patent claims methods of simultaneously introducing at least two streams of aqueous fluids through an inlet that is in fluid communication with a main channel through which oil is flowing. Each aqueous fluid comprises a reagent for conducting the reaction. *See, e.g.*, '091 patent, claim 1. Plugs are formed when the aqueous fluid is introduced from an inlet into the main channel and the oil partitions the aqueous fluid. *See, e.g.*, '091 patent, claim 1. Figure 2A of the '091 patent illustrates this process:



130. The claims further require that each plug comprises both the first and second reagent and that the reaction between the two reagents substantially occurs in the plug. '091 patent, claim 1.

5. '083 Patent

131. The '083 patent is directed generally to "microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid." '083 patent, at Abstract.

132. Generally, the claims of the '083 patent are directed to microfluidic systems and methods of conducting reactions in microfluidic systems where the systems comprise a non-fluorinated microchannel, a carrier fluid comprising a fluorinated oil and a fluorinated surfactant, at least one plug in the microchannel encased by the carrier fluid, where the surface tension at the plug-fluid/microchannel wall interface is higher than the surface tension at the plug-fluid/carrier

fluid interface. '083 patent at 73:10-75:4.

133. Independent claims 1 and 31 are directed to a microfluidic system, as discussed above, where the “surfactant is present at a concentration such that the surface tension at the plug-fluid/microchannel wall interface is higher than the surface tension at the plug-fluid/carrier fluid interface. Independent claim 20 is a method of conducting an autocatalytic reaction in such plugs.

B. Claim Construction

134. I understand that the Court has issued a claim construction order regarding certain terms of the patents at issue, and that the parties have agreed on the construction of certain additional terms of the patents at issue. See below for a summary of these claim constructions.

Claim Term	Construction
“autocatalytic reaction”	A reaction in which a product of the reaction is also a reagent for the same reaction.
“fluorinated compound”	A compound that includes one or more fluorine atoms.
“fluorinated oil”	An oil that includes one or more fluorine atoms.
“fluorinated surfactant”	An agent that reduces the surface tension between two liquids or a liquid or a solid, which includes one or more fluorine atoms.
“fluorosurfactant”	An agent that reduces the surface tension between two liquids or a liquid or a solid, which includes one or more fluorine atoms.
“plug”	A volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible.

“plug-fluid”	A fluid in which a reaction can occur or that can participate in a reaction.
“surfactant”	An agent that reduces the surface tension between two liquids or a liquid and a solid.
“microfluidic system”	System comprised of at least one substrate having a network of channels of micrometer dimension through which fluid may be transported. A “microfluidic system” is not limited to or the equivalent of a “substrate.”
“reagent”	Component of a plug-fluid that undergoes or participates in at least one type of reaction to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions. The location of reactions involving plug-fluids is not limited to the substrate.
“reaction”	Physical, chemical, biochemical or biological transformation.” The location of reactions is not limited to the substrate.
“providing conditions suitable for [the autocatalytic reaction/the reaction/a polymerase-chain reaction]”	providing a set of physical and chemical conditions that allow the [autocatalytic reaction/reaction/polymerase-chain reaction] to occur.
“A method for conducting an autocatalytic reaction in plugs in a microfluidic system”/’A method for conducting a reaction in plugs in a microfluidic system” (’193 and ’407 patents)	The entirety of the preambles are not limiting. The terms “reaction” and “microfluidic systems” are limiting and have the meanings set forth above.
“a single target DNA or RNA molecule”	One and only one target DNA or RNA molecule.
“Poisson distribution”	Distribution of target DNA or RNA molecules in plugs where there is an equal and independent probability for each target DNA or RNA molecule to be distributed into any one of a number of plugs.
“non-fluorinated microchannel”	Microchannel that is not composed of a material that includes fluorine atoms or that is treated to include fluorine atoms at its surface

	(excluding the possible exclusion of impurities or contaminants).
“biological molecule”	Molecules such as proteins, DNA, RNA, carbohydrates, and sugars. A biological molecule need not be naturally occurring.
“polymerase chain reaction”	Method of amplifying a target sequence of nucleic acids that involves repeated cycles of DNA replication, wherein 1) strands of DNA are denatured to form single-strand templates; 2) the templates are treated with oligonucleotide primers and a polymerase enzyme is used to extend the primers to produce replicated double-stranded DNA; 3) the replicated DNA then serves as a template for additional replication. The target sequence need not be specifically identified as a target in advance of the reaction.”

VIII. PRIOR ART

A. Quake

135. Quake, S., et al., “Microfabricated Crossflow Devices and Methods,” U.S. Patent Application Publication 2002/0058332 A1 (“Quake” or “Quake ’332 patent publication”) was published on May 16, 2002 from application No. 09/953,103 (the “Quake ’103 application”). The Quake ’103 application was filed on September 14, 2001, and claims priority to provisional application No. 60/246,793, filed on November 8, 2000, and provisional application No. 60/233,037 (the “Quake ’037 provisional application”), filed on September 15, 2000. I understand that Quake qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) at least as of September 14, 2001 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

136. I understand that Bio-Rad does not currently assert that any of Ismagilov's alleged inventions were conceived on or before September 14, 2001. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception (and I understand that 10X's position is that it should not be permitted), I reserve the right to rely on the Quake '037 provisional application which qualifies as prior art under 35 U.S.C. § 102(e) as of September 15, 2000. As set forth in 10X Genomics, Inc.'s Second Supplemental Initial Paragraph 4(d) Disclosure with Appendices A-I and Exhibits 38-54 (served July 21, 2017), which I incorporate by reference, many of the disclosures in Quake that I rely upon are also present in the Quake '037 provisional application, and these disclosures in the '037 provisional support published claims of Quake.

137. Quake described "[a] microfluidic device for analyzing and/or sorting biological materials." Quake at Abstract. In Quake's microfluidic system, droplets form when a continuously flowing aqueous solution contacts a continuously flowing oil. Quake at [0003] ("The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed . . . such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets."); *see also* Quake at [0315] ("The device comprises a main channel through which a pressurized stream or flow of a first fluid (e.g., oil) is passed");⁵ Quake at [0084] ("Preferably, the sample inlet intersects the main channel such that the pressurized sample solution is introduced into the main

⁵ As explained in more detail below, this sentence in Quake is almost directly copied in the Ismagilov provisional application (U.S. Provisional Application No. 60/379,927). *Compare* Quake at [0315] *to* the '927 provisional application at 32:15-16.

channel at an angle perpendicular to a stream of fluid passing through the main channel.”).⁶ Quake additionally taught that the carrier fluid could include a surfactant (or fluorinated surfactant) additive. Quake at [0300]. Quake made clear that the droplets disclosed in the application could be used for a variety of applications, including to conduct reactions. Quake at [0080]. While certain embodiments disclosed merging plugs containing different reagents as a method of conducting reactions, other embodiments described the presence of multiple reagents in a single fluid, so that all reagents necessary for a reaction were present at the time that the plug was formed. Quake at [0170]. For example, the specification states:

In another embodiment cells may produce a reported in vivo (e.g., a fluorescent compound) through interaction with a *reagent added to the fluid medium*. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g., benzene or naphthalene) with the net result that that fluorescence or another detectable property of the substrate will change.

Id. (emphasis added). Quake also explained that the microfluidic droplets could be used to conduct PCR. *See* Quake at [0080] (emphasis added) (“Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.”). Quake’s system thus used intersecting carrier fluid and aqueous fluid to form droplets, which could be used as microreactors to conduct different types of reactions, including PCR and other reactions with biological molecules.

138. Quake further taught that the concentration of reagents in droplets could be varied, so that reactions could take place in droplets. For example, Quake describes that “the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two

⁶ Likewise, this sentence in Quake is also almost directly copied in the Ismagilov provisional application. *Compare* Quake at [0084] to the ’927 provisional application at 31:2-4.

or more molecules, cells or virions.” Quake at [0120]. Though Quake described varying concentration⁷ for the purpose of sorting droplets, a POSA would have understood that Quake’s disclosures regarding concentration could be equally applied to the reactions Quake also described.

139. Further, Quake’s “droplets” clearly fall within the scope of the definition for “plugs” for the Ismagilov patents. I understand that the parties have agreed that the term “plug” means “a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible.” As described above, Quake’s droplets clearly fall within that definition.

140. Dr. Ismagilov himself understood that Quake described droplet formation using immiscible fluids in microfluidic devices. In a draft of a research article, Dr. Ismagilov wrote that “[t]his method takes advantage of spontaneous formation of droplets of immiscible fluid in microfluidic channels.[quake]” Ismagilov, R., et al., Draft Manuscript, “Microfluidic networks with rapid mixing and no dispersion (Pressure-driven plug transport in microchannels),” (RI00125397-125405) at RI00125397. Dr. Ismagilov therefore acknowledged that Quake had previously described “formation of droplets of immiscible fluid in microfluidic channels.” *Id.* He also wrote in the same draft that “Quake in his patent application (WO 02/23163 A1) at the very end had a figure (fig. 22) where he proposed to inject multiple aqueous reagents into a droplet.” RI00125401. And, Dr. Ismagilov clearly knew about the Quake ’332 patent publication, as he had a copy of Quake in his position. RI00121419.

141. Indeed, the Ismagilov technology is so similar to Quake—if not identical, in some cases—that the most logical conclusion is that substantial portions of the Ismagilov provisional

⁷ I note that none of the asserted patents require varying concentration as claim limitations.

application (U.S. Provisional Application No. 60/379,927) (“Ismagilov provisional” or “’927 provisional”) (RI00111580-636) that gave rise to all of the patents-in-suit were actually *directly copied* from the Quake PCT application (Quake, S., et al., “Microfabricated Crossflow Devices and Methods,” International Publication No. WO 02/23163 A1 (filed September 14, 2001; published March 21, 2002) (“Quake PCT”) (10X-000255470-595). This text is also included in Quake itself. For example, the following passage appears in the Quake PCT and Quake:

The device comprises a main channel through which a pressurized stream or flow of a first fluid (e.g., oil) is passed, and two or more inlet channels . . . which intersect the main channel at droplet extrusion regions . . . Preferably, these inlet channels are parallel to each other and intercept the main channel at a right angle. In specific embodiments wherein the droplets introduced through the different extrusion regions are mixed, the inlet channels are preferably close together along the main channel. For example, the main channel will typically have a diameter of 60 μm , that tapers to 30 μm at or near the droplet extrusion regions. The inlet channels also preferably have a diameter of about 30 μm and, in embodiments where droplet mixing is preferred, are separated by a distance along the main channel equal to approximately the diameter of the inlet channel (i.e., about 30 μm).

Quake PCT at 85:14-25; Quake at [0315]. This passage from the Quake PCT is almost *exactly* copied in the Ismagilov provisional, with the exception that the Ismagilov provisional replaces “droplet extrusion region” with “plug-forming region.” But as explained above, Quake’s droplets fall within the construction of “plug.” The same paragraph from the Ismagilov provisional appears below:

[A] substrate according to the invention may **comprise a first channel through which a pressurized stream or flow of a carrier-fluid is passed, and two or more inlet channels which intersect the first at plug-forming regions . . . Preferably, these inlet channels are parallel to each other at a right angle. In**

specific embodiments wherein the plugs introduced through the different plug forming regions are mixed, the inlet channels are preferably close together along the first channel. For example, the first channel will typically have a diameter of 60 μm , that tapers to 30 μm at or near the plug-forming regions. The inlet channels then also preferably have a diameter of about 30 μm and, in embodiments where droplet mixing is preferred, are separated by a distance along the first channel approximately equal to the diameter of the inlet channel (i.e., about 30 μm).

'927 provisional application at 15:62-16:11 (emphasis added). The portions of the Ismagilov provisional that are closely or directly copied from the Quake PCT are bolded and underlined above. This copying of the Quake PCT by Ismagilov underscores how similar—and indeed, in parts, identical—the two patents are to each other. There are at least 43 passages in the Ismagilov provisional that are substantially or directly copied from the Quake PCT, many of which span multiple paragraphs. I have attached a chart that compares all of these paragraphs, as well as similar language in the '407 patent, as **Exhibit 3** to this report. The language that is repeated from the Quake PCT and Quake is bolded and underlined.

142. As another example of the similarities between the Quake PCT/Quake and Ismagilov, Ismagilov's description of a "detection region" is almost exactly copied from the Quake PCT. The Quake PCT's description of a "detection region" appears below:

A detection region is within, communicating, or coincident with a portion of the main channel at or downstream of the droplet extrusion region and, in sorting embodiments, at or upstream of the discrimination region or branch point. Precise boundaries for the detection region are not required, but are preferred.

Quake PCT at 25:28-26:2; Quake at [0086]. Again, the same description appears in the Ismagilov provisional application, changing "main channel" to "first channel" and "droplet extrusion region" to "plug-forming region:

A detection region is within, communicating, or coincident with a portion of a first channel at or downstream of the plug-forming region and, in sorting embodiments, at or upstream of the discrimination region or branch point. Precise boundaries for the detection region are not required, but are preferred.

'927 provisional application at 31:18-21. The entirety of this paragraph in the Ismagilov provisional application is almost directly copied from the Quake PCT and Quake.

143. The similarities between Quake and Ismagilov—which appear to be a direct result from the copying of Quake's PCT application into the Ismagilov provisional application—serve to demonstrate how Quake anticipates or renders obvious the claims of the Ismagilov patents, as explained below.

144. The amount of apparent copying is striking when looking at the Ismagilov provisional application as a whole. The fact that Ismagilov apparently copied so directly from the Quake PCT illustrates how similar Quake and the Ismagilov patents actually are. As I explain in greater detail below, it is my opinion that Quake either anticipates or renders obvious all claims of the asserted Ismagilov patents. As part of my invalidity analysis, I have considered the portions of the Ismagilov provisional apparently copies from Quake.

145. I understand that during prosecution of the Ismagilov '407 and '193 patents, the examiner characterized Quake using the following language:

Quake and Thorsen in US 2002/0058332 (IDS) disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil; however, the droplets in the oil are not formed from two continuously flowing immiscible fluids, but rather by a discrete dispensing the solution with biological material into the carrier fluid. This is a different approach from the claimed invention.

'407 Prosecution History, Notice of Allowability (dated August 8, 2012) (RDTX00002972-76)

at 4; '193 Prosecution History, Notice of Allowability (dated August 7, 2012) (RDTX00002243) at 3-4.

146. I disagree with the examiner's characterization of Quake. Quake clearly describes two continuously flowing fluids. For example, Quake describes that "[t]he devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which *a pressurized stream of aqueous solution is passed*." Quake at [0003] (emphasis added); *see also* Quake at [0015] ("A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel."); Quake at [0070] (emphasis added) ("An 'extrusion region' or 'droplet extrusion region' is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel."); Quake at [0115] ("The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.").

147. As another example, Quake also describes that "[i]n preferred embodiments, a first fluid, which may be referred to as an 'extrusion' or 'barrier' fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a 'sample' or 'droplet'

fluid, passes or flows through the inlet region.” Quake at [0020].

148. Quake also made clear that the “flow” of the aqueous fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining *a flowing stream of an aqueous solution* and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Prosecution History of U.S. Patent Application No. 09/953,103, Amendment (dated September 25, 2006) (“Quake ’103 Response”) (10X-000255448-69)) at 15 (emphasis added).

B. Shaw Stewart

149. Shaw Stewart, P., “Combining chemical reagents,” UK Patent Application Publication GB 2,097,692 A (“Shaw Stewart”) was published on November 10, 1982 from application No. 8200642. I understand that Shaw Stewart qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of November 10, 1982 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” Pre-AIA 35 U.S.C. § 102(b).

150. Shaw Stewart described “a method of combining chemical reagents” where the reagents are dispensed in “discrete volumes of droplets separated from each other by an inert immiscible liquid.” Shaw Stewart at Abstract. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier flows down the tube. When each droplet almost spans the tube, it will be broken off.” Shaw Stewart at 1:83-88.

151. Shaw Stewart additionally described that his system was “particularly suited to

the manipulation of microscopic quantities of reagents, with volumes of less than one microlitre . . .” Shaw Stewart at 1:20-22. Though Shaw Stewart does not elaborate further on the lower limit of the volumes that could be used with his invention, he did describe the same system in a later PCT application. In the PCT application, which also serves as prior art to the Ismagilov patents (“Shaw Stewart II”), Shaw Stewart further described that the *same* system⁸ described in the Great Britain patent application was “particularly suited to the manipulation of microscopic quantities of reactant with volumes of less than 10 nanolitres.” Shaw Stewart II at 3:18-19. Bio-Rad has admitted that these dimensions “disclose[] a ‘microfluidic product.’” IPR2015-00009 Petition at 8. Therefore, Shaw Stewart disclosed a microfluidic droplet system in which reactions, including reactions with biological molecules, could be conducted.

152. Further, Shaw Stewart’s “droplets” clearly fall within the scope of the definition for “plugs” for the Ismagilov patents. I understand that the parties have agreed that the term “plug” means “a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible.” As described above, Shaw Stewart’s droplets clearly fall within that definition.

153. Indeed, Dr. Ismagilov himself acknowledged that Shaw Stewart describes plugs. In a draft of a research article, he noted that in Shaw Stewart’s patent application (referencing Shaw Stewart’s GB patent application discussed in this report), “Stewart . . . has shown that plugs of individual reagents can be made and then merged to initiate a reaction.” RI00125402.

⁸ Bio-Rad, in a prior proceeding, explained that “Stewart I [the Shaw Stewart Great Britain patent application] & Stewart II [the Shaw Stewart PCT application] both disclose the exact same droplet formation mechanism used by [a Quake patent], and Stewart II discloses further details about smaller droplet sizes, and hence smaller channel sizes, used in this prior art device.” IPR2015-0009 Petition at 2.

C. Burns (2001)

154. Burns, J.R. and Ramshaw, C., “The intensification of rapid reactions in multiphase systems use slug flow in capillaries,” *Lab Chip*, 1:10-15 (2001) (“Burns (2001)”) was “[f]irst published as an Advance Article on the web 9th August 2001.” Burns (2001) at 10. I understand that Burns (2001) qualifies as prior art under pre-AIA 35 U.S.C. § 102(a) as of August 9, 2001 because “the invention was . . . described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.” 35 U.S.C. § 102(a).

155. In 2001, J.R. Burns and C. Ramshaw from the University of Newcastle described a “multiphase microreactor based upon the use of slug flow through a narrow channel.” Burns (2001) at 10. These “slugs” were formed by “the continuous flow of both [liquid] phases through T or cross-shaped intersections.” *Id.* at 11. Burns (2001) described that slugs were “generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting of the flow of the first phase into the channel and reversing the process.” *Id.* Burns (2001) further described that the “slugs” could be used as “microreactors.” Burns (2001) at Abstract. Burns (2001) describes a simple acid-base reaction between acetic acid and NOH and acetic acid and KOH in these plugs. *Id.* at 11. The progress of this reaction was monitored by photographing color change. *Id.* Therefore, Burns (2001) described reactions within plugs to be controlled by mass transfer. They report that “not only is mass transfer between slugs enhanced by internal circulation to beyond that expected for diffusion at millimeter slug length scales, but to level as good as that expected from diffusion between two parallel flow streams.” Burns (2001) at 14. They hence conclude reactions in slug flow is superior reactions in parallel (or laminar) flow.

156. Though Burns (2001) referred to the encapsulated fluid as “slugs” and stated that “[b]ased on experimental observation of the slug flow there appeared to be no liquid layer

surrounding the slugs within the resolution of the photography, which was well below 25 μm ,” *id.* at 13, an image of the “slugs” provided in the publication shows otherwise:

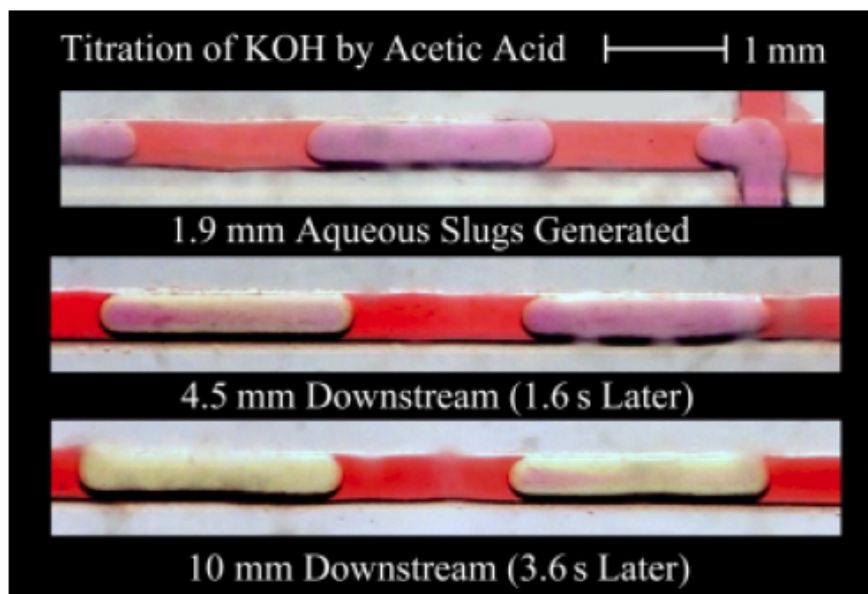


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns at Fig. 4.

157. Burns describes that “[p]henol Red pH indicator was added to distilled water . . . This was then used to produce aqueous solutions of KOH and NaOH . . . Sudan III(red) . . . dye [was] added [to the organic phase with 67% silicone oil] to aid flow visualization.” Burns at 11. Acetic acid in the organic oil transfers to the water phase and “the pH indicator in the aqueous phase changed from pink to yellow.” Burns at 11. The color change can clearly been seen in the aqueous “slugs” in the image above. Importantly, a thin film of the red organic oil is evident below the aqueous “slugs” throughout the image. Since their capillary dimension of 380 microns is too small for gravity to become important and the “top” and “bottom” of the channel is symmetric, a similar film should exist above the “slug” even if it does not appear in the image due to camera angle. Based on my experience and the image above, it is my opinion that the

“slugs” described in Burns (2001) are “plugs” that are fully or substantially encapsulated by the organic phase. Therefore, Burns (2001) disclosed a microfluidic *plug* system in which reactions, including chemical reactions, could be conducted.

158. Further, Burns’s “slugs” clearly fall within the scope of the definition for “plugs” for the Ismagilov patents. I understand that the parties have agreed that the term “plug” means “a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible.” As described above, Burns’s slugs clearly fall within that definition.

159. Indeed, Dr. Ismagilov himself characterized Burns’s “slugs” as “plugs.” For example, in an article co-authored by Dr. Ismagilov and entitled “Reactions in Droplets in Microfluidic Channels,” Dr. Ismagilov wrote that “Organic reactions have been performed in microfluidic plugs and slugs to exploit the advantages of miniaturization as well as facile heat and mass transfer. Examples of single-step reactions that have been performed within plugs include . . . the extraction of acid from kerosene [citing Burns (2001)].” Song, H., et al., “Reactions in Droplets in Microfluidic Channels,” *Angew. Chem. Int. Ed.* 2006 (RI00139536-139556). Though Dr. Ismagilov expressly distinguished “slugs” from “plugs,” he went on to explicitly describe Burns (2001) as disclosing “plugs.” Therefore, Dr. Ismagilov himself characterized Burns (2001) as describing plugs.

D. Nisisako

160. Nisisako, T., et al., “Droplet formation in a microchannel network,” *Lab Chip*, 2:24-26 (2002) (“Nisisako”) was “[f]irst published as an Advance Article on the web 18th January 2002.” Nisisako at 24. I understand that Nisisako qualifies as prior art under pre-AIA 35 U.S.C. § 102(a) as of January 18, 2002 because “the invention was . . . described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.”

35 U.S.C. § 102(a).

161. Nisisako discloses “[a] method is given for generating droplets in a microchannel network. With oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep. For given experimental parameters, regular-sized droplets are reproducibly formed at a uniform speed.” Nisisako at Abstract.

162. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26. Therefore, Nisisako disclosed a microfluidic droplet system in which reactions, including biological reactions, could be conducted.

163. Further, Nisisako’s “droplets” clearly fall within the scope of the definition for “plugs” for the Ismagilov patents. I understand that the parties have agreed that the term “plug” means “a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible.” As described

above, Nisisako's droplets clearly fall within that definition.

E. Thorsen

164. Thorsen, T., et al., "Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device," *Physical Review Letters*, 86(18): 4163-4166 (2001) ("Thorsen") was published on April 30, 2001. I understand that Thorsen qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of April 30, 2001 because "the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States." 35 U.S.C. § 102(b).

165. Thorsen described the formation of droplets "by shearing one liquid into a second immiscible one, often in the presence of a surfactant." Thorsen at 4163; *see also id.* ("[W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets."). These droplets "may . . . find application as a component of a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds." *Id.* at 4166. Therefore, Thorsen disclosed a microfluidic droplet system in which reactions, including reactions involving biological molecules, could be conducted.

166. Further, Thorsen's "droplets" clearly fall within the scope of the definition for "plugs" for the Ismagilov patents. I understand that the parties have agreed that the term "plug" means "a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible." As described above, Thorsen's droplets clearly fall within that definition.

F. Seki

167. Seki, M., et al., “Control Mechanism for Trace Quantity of Liquid,” U.S. Patent Application Publication 2002/0195463 A1 (“Seki”) was published on December 26, 2002 from U.S. Patent Application No. 10/157,075 (the “Seki ’075 application”). The Seki ’075 application was filed on May 30, 2002, and claims priority to foreign application 2001-163740 (JP), filed on May 31, 2001. I understand that Seki qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) as of May 30, 2002 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

168. Seki describes a “control mechanism for a trace quantity of liquid . . . comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flowchannel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

169. Seki further describes that reactions can be conducted in these droplets. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145]. Seki thus disclosed a microfluidic droplet system in which reactions,

including reactions involving biological molecules, could be conducted.

170. Further, Seki's droplets clearly fall within the scope of the definition for "plugs" for the Ismagilov patents. I understand that the parties have agreed that the term "plug" means "a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible." As described above, Seki's droplets clearly fall within that definition.

G. Corbett

171. Corbett, J., et al., "Device and Method for the Automated Cycling of Solutions Between Two or More Temperatures," U.S. Patent No. 5,270,183 ("Corbett") issued on December 14, 1993. I understand that Corbett qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of December 14, 1993 because "the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States." 35 U.S.C. § 102(b).

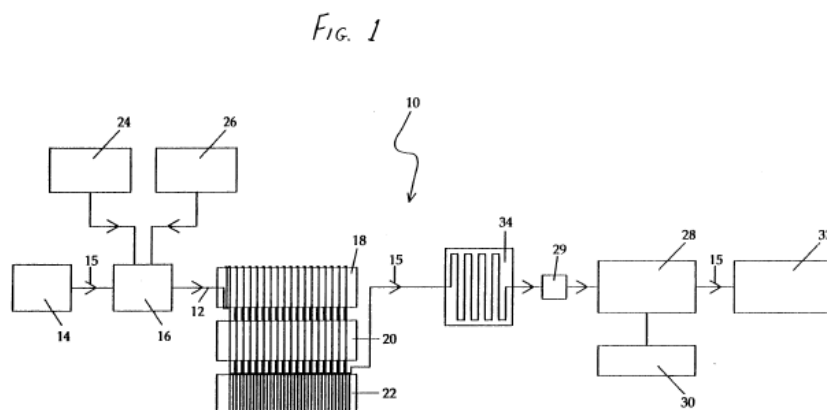
172. Corbett describes "an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction" by injecting "a reaction mixture into a stream of carrier fluid. The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place." Corbett at Abstract. Specifically, Corbett describes that one aspect of the invention comprises a "method of cyclically heating and cooling a reaction mixture comprising:

- i) injecting the reaction mixture into a stream of carrier fluid in which the reaction mixture is immiscible;
- ii) bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a pre-determined period of time;

- iii) repeating step 2 until the desired number of heating and cooling cycles have been achieved; and
- iv) recovering the reaction mixture from the carrier fluid.

Corbett at 3:24-37.

173. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that the reaction mixture “compris[es] the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components,” and the “small volume [of reaction mixture], approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Corbett thus describes a PCR apparatus which uses immiscible fluids to isolate reaction mixtures.

H. Lagally

174. Lagally, E.T., et al., “Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device,” *Analytical Chemistry*, 73(3): 565-570 (2001) (“Lagally”)

published on February 1, 2001. I understand that Lagally qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of February 1, 2001 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

175. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, the authors describe “the development of a monolithically integrated PCR-CE device . . . [which includes] 280-nL PCR chambers etched into a glass substrate, connected to microfluidic valves and hydrophobic vents for sample introduction and immobilization during thermal cycling.” Lagally at 566. Further, Lagally discloses “an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Therefore, Lagally disclosed PCR in a microfluidic device.

I. Burns (1996)

176. Burns, M., et al., “Microfabricated structures for integrated DNA analysis,” *Proc. Natl. Acad. Sci. USA*, 93: 5556-5561 (1996) (“Burns (1996)”) published May 28, 1996. I understand that Burns (1996) qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of May 28, 1996 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

177. Burns (1996) describes performing microfluidic chip PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Figure 1 of Burns (1996) is reproduced below:

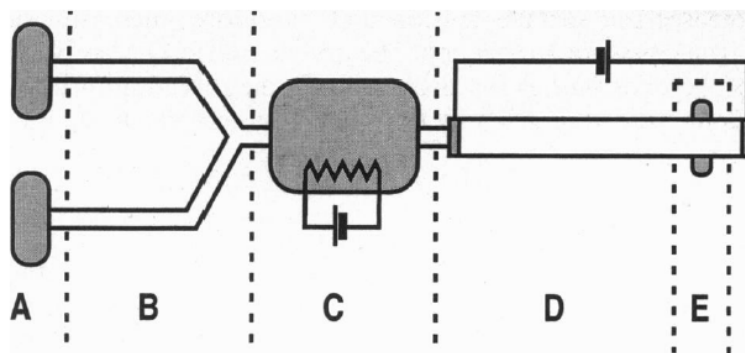


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

Burns (1996) at Fig. 1. Burns (1996) further describes that “[t]he microfabricated elements in this report are capable of performing several processing steps in conventional DNA analysis. The individual elements have the potential for combination into a complete DNA genotype analysis processing path. No new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” Burns (1996) at 5560. Therefore, Burns (1996) disclosed a microfluidic chip used to perform PCR.

J. Curcio

178. Curcio, M., “Improved Techniques for High-Throughput Molecular Diagnostics,”

Ph.D. Thesis, Department of Chemistry Division of Analytical Chemistry, Royal Institute of Technology SE-100 44 Stockholm Sweden (2002) (“Curcio”) was made available online on September 11, 2002 through the DiVA publishing system. Curcio, M., “Improved Techniques for High-Throughput Molecular Diagnostics,” Doctoral Thesis, Abstract (<http://www.diva-portal.org/smash/record.jsf?pid=diva2%3A9192&dswid=4823#sthash.TxMAotEx.dpbs>) (10X-000255216). I understand that Curcio qualifies as prior art under pre-AIA 35 U.S.C. § 102(a) as of September 11, 2002 because “the invention was . . . described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.” 35 U.S.C. § 102(a).

179. Curcio describes “[a] continuous segmented-flow method for sequential DNA amplification” using a “15 meter long narrow-bore Teflon tube, coiled such as to be repeatedly exposed to three different temperature zones, optimized according to a PCR protocol.” Curcio at Paper V, 1. An exemplary device is depicted in Figure 1 reproduced below:

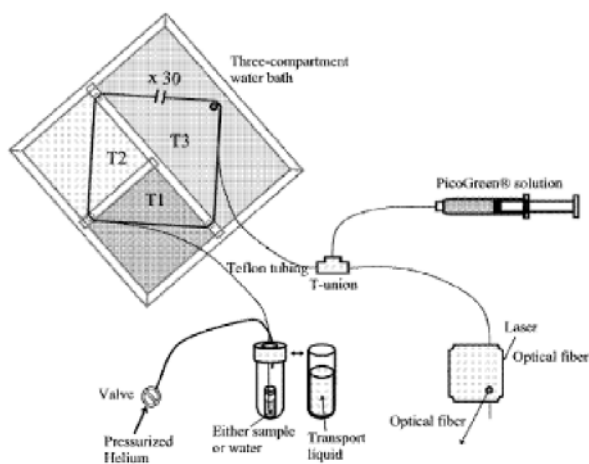


Figure 1.

180. “To minimize carry-over, the aqueous samples travel as separate segments in a continuous flow of immiscible organic liquid.” *Id.* Curcio describes that perfluorodecalin was

utilized as a carrier fluid because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity towards biomolecules.” *Id.* at 9. The following conditions for performing PCR are described:

PCR. The tube, 15 m length from inlet to outlet, was passed 30 times through the three temperature zones, thus corresponding to 30 PCR cycles. The tube length per cycle was 44 cm with a ratio 1:1:2 for the three steps, hence 11 cm for the denaturing step (33 cm for the first cycle), 11 cm for the annealing step and 22 cm for the elongation step (66 cm for the last cycle).

As DNA source, genomic human placenta DNA (Sigma-Aldrich, Saint Louis, Missouri, USA) was utilized at a concentration of 5 ng/J..Ll. A 79 bp region of the A1AT (alpha 1-antitrypsin) gene and a 148 bp region of the CFTR (cystic fibrosis transmembrane conductance regulator) gene were used as targets for amplification. Primers (purchased from Thermo Hybaid, Ulm, Germany), used at a concentration of 0.4 J..LM each, were: 5'-TGCATAAGGCTGTGCTGACC-3' and 5'AGACATGGGTATGGCCTCT- 3' for A1AT, while for CFTR these were 5'ACTGGAGCCTTCAGAGGGTAAAT -3' and 5'-ATGCTTTGATGACGCTTCTG-3'. AmpliTaq polymerase (Sigma-Aldrich, Saint Louis, Missouri, USA) was used in 0.02 units per reaction while the concentration of each dNTP (Sigma-Aldrich, Saint Louis, Missouri, USA) 200 J..LM in a total reaction volume of approximately 300 nl. This volume corresponded to a sample plug length of ca 1 cm.

The temperatures in the three compartments were set as follows: denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C. The flow rate in the tubing determines the residence time in each zone. The experiments were performed with a time frame of 90 seconds per cycle. Two negative controls were also used in the experiments: one contained all the reagents at the same concentration as for the

normal reaction mixtures, however without DNA template; another contained the DNA template but no DNA polymerase. All the reaction mixtures were freshly prepared on a daily basis and kept at 0 °C before use.

Id. at 5. Curcio described the successful amplification of a sequence of samples as well as the successful amplification of a 148 bp portion of the CFTR gene. *Id.* at 9. “In order to allow fluorescence detection of the amplified material, PicoGreen® was added to the sample plugs by means of flow injection via a T-union.” *Id.* Curcio explains that “[m]initurization of the fluidic system is beneficial in two ways: it enhances the speed of thermal equilibration of the reaction mixture, thus allowing increased flow velocities and faster PCR. Also analyte volumes are reduced, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level. *Id.* at 7. Curcio thus disclosed a system for conducting PCR using segments of aqueous fluid in a continuous flow of a fluorinated carrier fluid.

K. Anderson

181. Anderson, B., et al., “Chemical Amplification Based on Fluid Partitioning,” U.S. Patent No. 7,041,481 B2 (“Anderson”) issued on May 9, 2006 from application No. 10/389,130, filed on March 14, 2003. I understand that Anderson qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) as of March 14, 2003 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

182. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

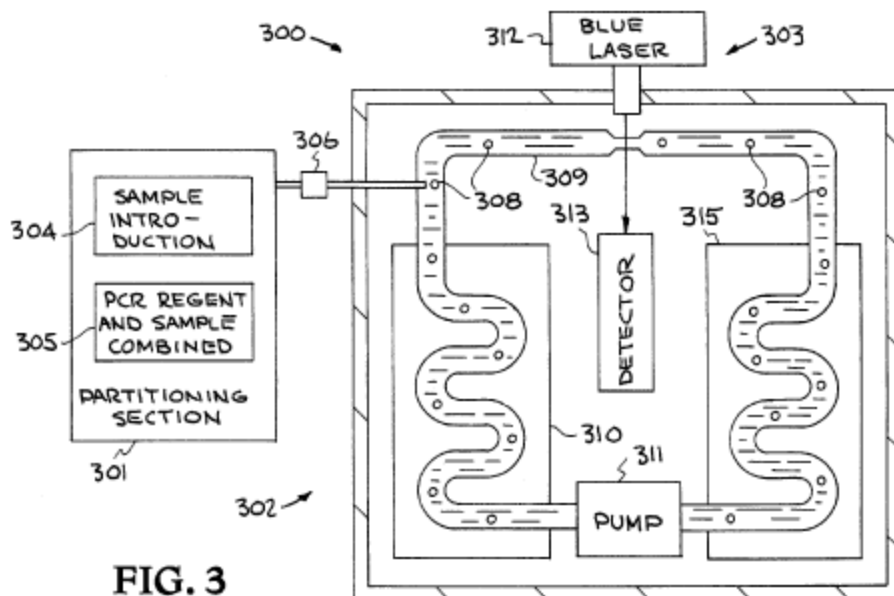


FIG. 3

183. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” *Id.* at 7:47-50. Further, “given the extremely small volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” *Id.* at 7:35-36. Therefore, Anderson disclosed a system for conducting PCR in microdroplets. Anderson describes multiple advantages to such a system:

Isolating the PCR reaction in such small (picoliter) volumes provides an order of magnitude reduction in overall detection time by:

(1) reducing the duration of each temperature cycle—the concentration of reactants increases by enclosing them in picoliter type volumes. Since reaction kinetics depend on the concentration of the reactant, the efficiency of a microdroplet should be higher than in an ordinary vessel (such a test tube) where the reactant quantity is infinitesimal

(2) reducing the total number of cycles—dilution of the fluorescently generated signal is largely eliminated in such a small volume, allowing much earlier detection. This effect is directly related to the number of microdroplets formed from the initial sample/reagent pool. Since PCR is an exponential process, for

example, 1000 microdroplets would produce a signal 10 cycles faster than typical processing with bulk solutions.

(3) removing interference from competing DNA templates—given the extremely small volumes involved, it is possible to isolate a single template of the target DNA in a given microdroplet. A pL microdroplet filled with a 1 pM solution, for example, will be occupied by only one molecule on average. This makes it possible to amplify only one template in mixtures containing many kinds of templates without interference. This is extremely important in processing of real world aerosol samples containing complex mixtures of DNA from many sources, and has direct application in screening of precious cDNA libraries.

Id. at 7:63-8:25.

L. Wang

184. Wang, L., et al., “Method and Apparatus for Production of Small Particles of Micrometer or Nanometer Size,” International Publication No. WO 00/23181 (“Wang”) published on April 27, 2000, designating the United States. I understand that Wang qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) as of April 27, 2000 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

185. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution and a precipitating solution together in a confined reaction zone.” Wang at Abstract. In one embodiment, “the method is performed by using a Flow Injection Synthesis (FIS) apparatus to be able to carry out the reaction in confined reaction zones, such as droplets, where agglomeration and aggregation can be limited.” *Id.* at 2:4-6. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. *Id.* at 2:19-26. These small particles are “micrometer or nanometer size.” *Id.* at 1:4-5. An advantage of this

system is that “the synthesis is carried out via reactions in solution in confined zones, which are separated from each other by aqueous, non-aqueous, organic or gas phase. The particles as formed in the individual reaction zones are not brought in contact with other particles in other reaction zones.” *Id.* at 3:26-30. Therefore, Wang disclosed a method for conducting reactions within micrometer- or nanometer-sized particles.

M. Ramsey

186. Ramsey, J.M., et al., “Microfluidic Devices for the Controlled Manipulation of Small Volumes,” U.S. Patent No. 6,524,456 B1 (“Ramsey”) issued on February 25, 2003 from application No. 09/408,060, filed on September 29, 1999. I understand that Ramsey qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) as of September 29, 1999 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

187. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nano- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50. Indeed, Ramsey describes that “[t]he method and apparatus according to this invention have application to such problems as screening molecular or cellular targets using single beads from split-synthesis combinatorial libraries, screening single cells for RNA or protein expression, genetic diagnostic screening at the single cell level, or performing single cell signal transduction studies.” *Id.* at Abstract. Therefore, Ramsey

disclosed an apparatus for carrying out reactions in nano- to subnano-liter volumes, using fluorinated oils to separate the fluid volumes.

N. Schubert

188. Schubert, K.V. and Kaler, E.W., “Microemulsifying fluorinated oils with mixtures of fluorinated and hydrogenated surfactants,” *Colloids and Surfaces A: Physiochemical and Engineering Aspects*, 84: 97-106 (1994) (“Schubert”) published on April 18, 1994. I understand that Schubert qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of 1994 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

189. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at Abstract. Schubert notes that “[r]esearch on the formulation and structure of microemulsions containing fluorinated compounds has recently been active,” and that “[f]luorinated compounds . . . offer the potential for biomedical applications.” *Id.* at 97. This is because, for example, “fluorinated alkanes are . . . are chemically and biologically stable.” *Id.* “The fluorinated surfactants used are commercial blends of non-ionic *n*-polyglycol ethers with a perfluorinated alkyl chain . . . from DuPont (Zonyl FSO-100 . . . and Zonyl FSN-100 . . .).” *Id.* at 98. Therefore, Schubert disclosed microemulsions with an aqueous phase surrounded by a fluorinated oil and fluorinated surfactant.

O. Krafft

190. Krafft, M.P., et al., “Multiple Emulsions Comprising a Hydrophobic Continuous Phase,” U.S. Patent No. 5,980,936 (“Krafft”) issued on November 9, 1999 from application No. 08/908,821, filed on August 7, 1997. I understand that Krafft qualifies as prior art under pre-AIA

35 U.S.C. § 102(b) as of July 2, 1999 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

191. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58. Therefore, Krafft disclosed microemulsions with an aqueous phase surrounded by a fluorinated oil and fluorinated surfactant.

P. Kenis

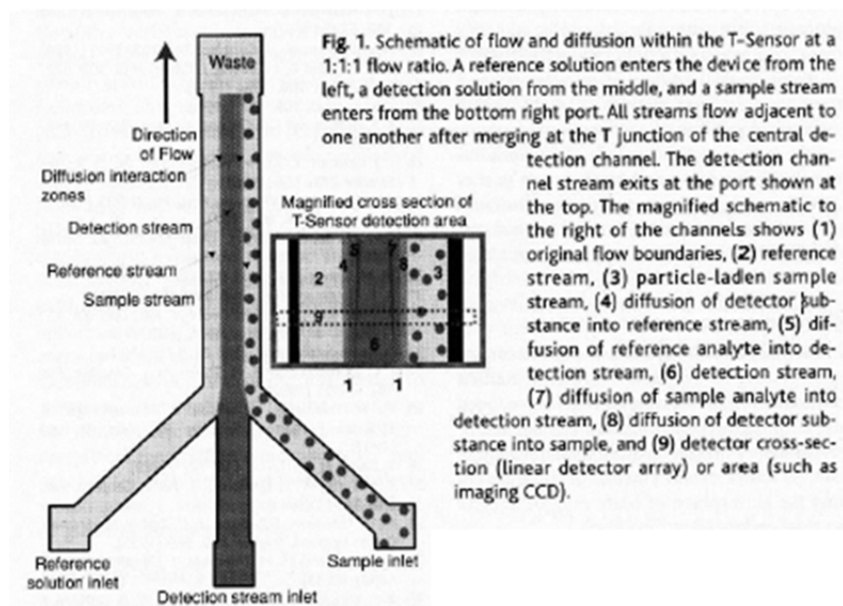
192. Kenis, P., et al., “Microfabrication Inside Capillaries Using Multiphase Laminar Flow Patterning,” *Science*, 285: 83-85 (1999) (“Kenis”) published on July 2, 1999. I understand that Kenis qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of July 2, 1999 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

193. Kenis describes a microfluidic system in which “multiple liquid streams flowing laminarly” are introduced in a single channel. Kenis at 83. The liquid stream are “reactive species” and “chemical reactions occur[] either between the streams and the interior surface of the capillary, or at the interface between the streams.” *Id.* Further, Kenis teaches that “[b]ecause many combinations of parallel fluid streams can be generated using “Y” or “T” junctions (or their extensions to multiple streams), it possible to bring a wide variety of solutions in contact with one another.” *Id.* Therefore, Kenis described a microfluidic system in which reactions could be conducted using two fluids in laminar flow.

Q. Weigl

194. Weigl, B.H. and Yager, P., “Microfluidic Diffusion-Based Separation and Detection,” *Science*, 283: 346-347 (1999) (“Weigl”) published on January 15, 1999. I understand that Weigl qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of January 15, 1999 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

195. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. “In these tiny microchips etched with grooves and chambers, a multitude of chemical and physical processes for both chemical analysis and synthesis can occur. These devices, also known as micro-total analysis systems (μ TAS) . . . offer many advantages over traditional analytical devices: They consume extremely low volumes of both samples and reagents.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including sampling, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). For example, Figure 1 of Weigl shows a schematic of this parallel fluid flow:



Weigl at Fig. 1. Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347. Therefore, Weigl described a microfluidic system in which reactions could be conducted using two fluids in laminar flow.

R. Kopf-Sill

196. Kopf-Sill, A., et al., “Microfluidic Systems Incorporating Varied Channel Dimensions,” U.S. Patent No. 5,842,787 (“Kopf-Sill”) issued on December 1, 1998 from application No. 948,194, filed on October 9, 1997. I understand that Kopf-Sill qualifies as prior art under pre-AIA 35 U.S.C. § 102(e) as of October 9, 1997 because “the invention was described in” a published “application for patent . . . by another filed in the United States before the invention by the applicant for patent.” Pre-AIA 35 U.S.C. § 102(e)(1).

197. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill at Abstract. The materials to be mixed are introduced in

parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16. For example, Kopf-Sill describes that “channels 514 and 516 intersect in main channel 518. The materials to be mixed, 504 and 506, are concomitantly introduced into main channel 518 from channels 514 and 516, respectively.” *Id.* at 14:6-10 (describing Figure 5B). Figure 5B of Kopf-Sill is reproduced below:

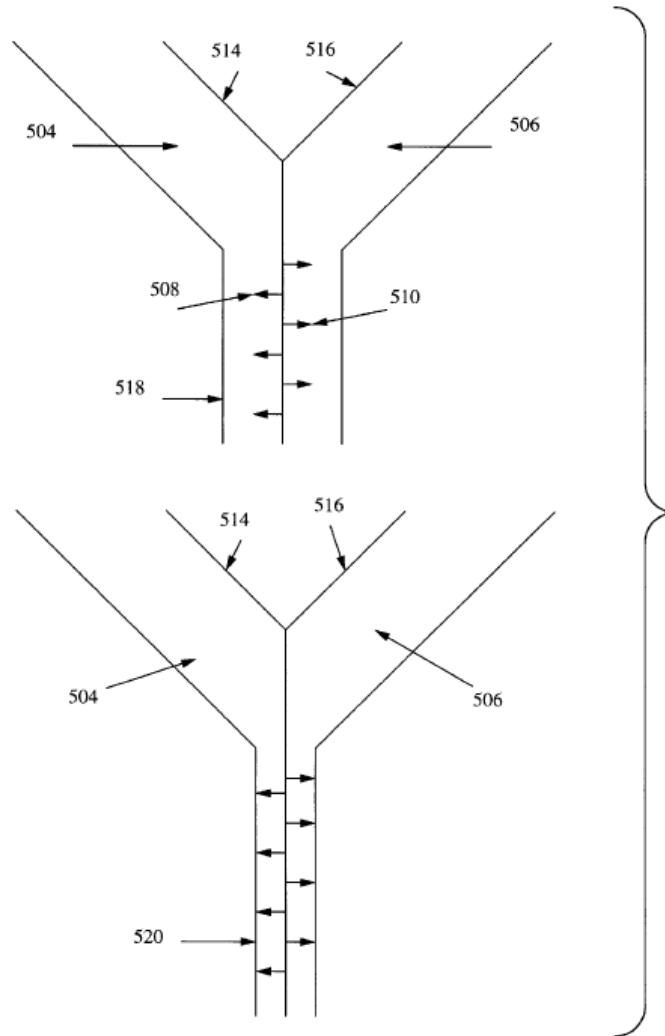


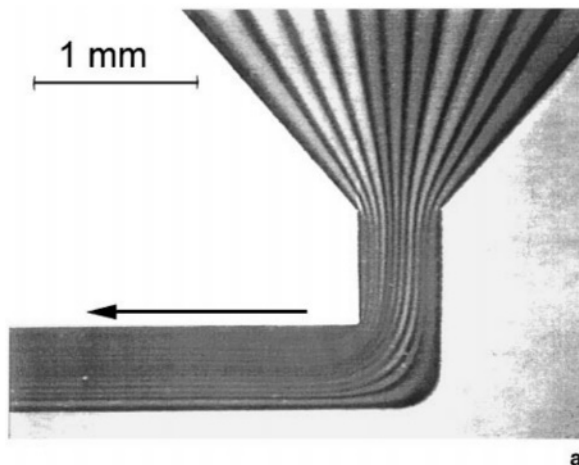
Figure 5B

Kopf-Sill at Fig. 5B. Therefore, Kenis described a microfluidic system in which reactions could be conducted using two fluids in laminar flow.

S. Erbacher

198. Erbacher, C., et al., “Towards Integrated Continuous-Flow Chemical Reactors,” *Mikrochim. Acta*, 131: 19-24 (1999) (“Erbacher”) published in January 1, 1999. I understand that Erbacher qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of January 1, 1999 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

199. Erbacher describes “[a] device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at Abstract. “As the main mechanism for mixing is diffusional mass transport, the flow has to be split into several laminae which are narrower than the capillary width.” *Id.* The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



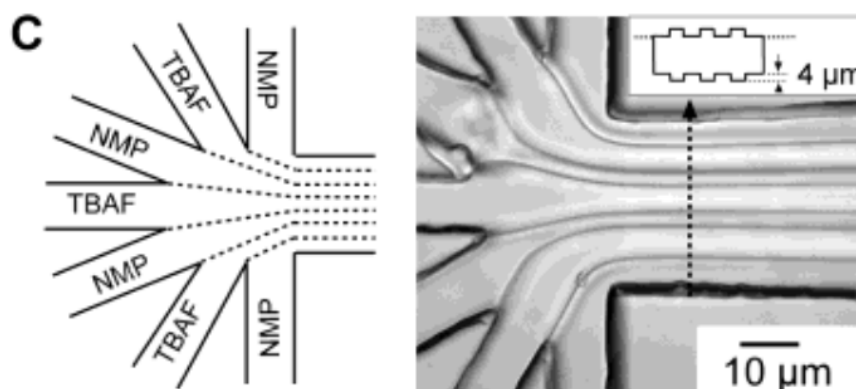
Erbacher at Fig. 4a. Therefore, Erbacher described a microfluidic system in which reactions could be conducted using two fluids or more in laminar flow.

T. Whitesides

200. Kenis, P., et al., “Fabrication inside Microchannels Using Fluid Flow,” *Accounts*

of Chemical Research, American Chemical Society, 33(11) (2000) (“Whitesides”) was “published on Web 09/08/2000” and was published in print on December 1, 2000. I understand that Whitesides qualifies as prior art under pre-AIA 35 U.S.C. § 102(b) as of September 8, 2000 because “the invention was patented or described in a printed publication in this or a foreign country . . . more than one year prior to the date of the application for patent in the United States.” 35 U.S.C. § 102(b).

201. Whitesides describes a continuous flow microchannel reactor that could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



Whitesides at 845-846. Therefore, Whitesides described a microfluidic system in which reactions could be conducted using two fluids in laminar flow.

IX. INVALIDITY OF THE '407 PATENT

A. Summary of the '407 Patent

202. The '407 patent is entitled "Method for Conducting Reactions Involving Biological Molecules in Plugs in a Microfluidic System." The abstract explains that the invention "provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid." '407 patent at Abstract.

203. I understand that Bio-Rad is asserting claims 1-5, 8-11 and 13 of the '407 patent. Of these claims, only claim 1 is independent. Claims 2, 5, 8, and 13 depend on claim 1. Claims 3 and 4 depend on claim 2. Claims 9 and 11 depend on claim 8, and claim 10 depends on claim 9.

204. The '407 patent issued from Application No. 13/024,145, filed February 9, 2011 (the "'145 application"). The '145 application was a continuation of application No. 12/777,099, filed on May 10, 2010, which was a continuation of application No. 10/765,718, filed on January 26, 2004, which itself was a continuation-in-part of application No. 10/434,970 (which issued as the '091 patent), filed on May 9, 2003.

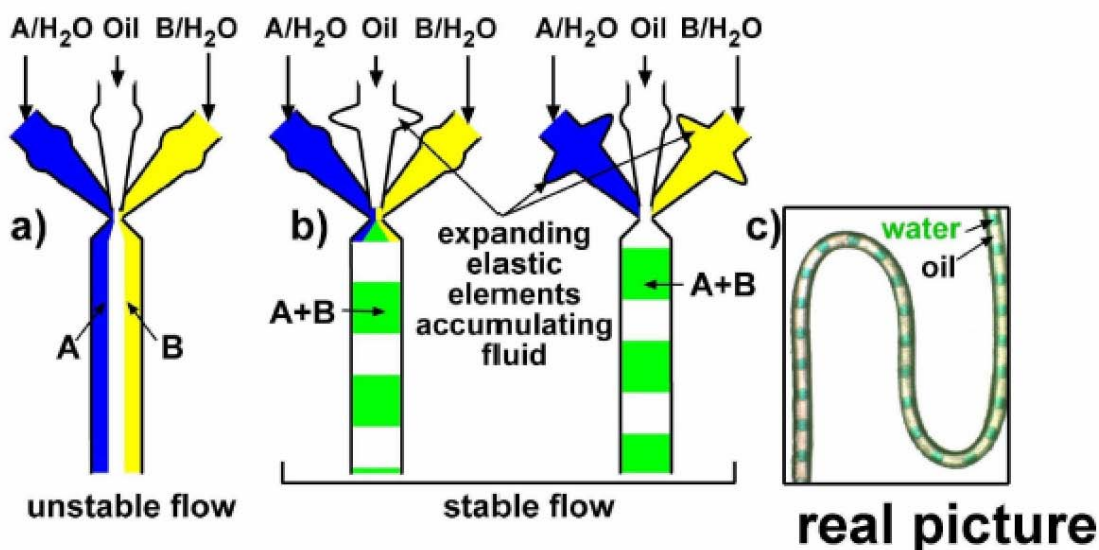
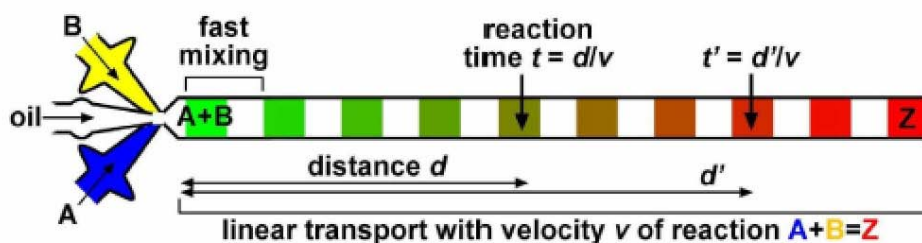
1. Priority

205. I understand that Bio-Rad asserts that claims 1-3, 5, 8-9, and 13 of the '407 patent were conceived of "no later than October 16, 2001," and relies on RI00111660-70 to support this assertion. Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 4. I further understand that Bio-Rad asserts that claim 4 was conceived of "no later than October 30, 2002," and relies on RI00111690-738 to support this assertion; that claim 10 was conceived of "no later than February 8, 2002," and relies upon RI00111308 and RI00111321 to support this assertion; and that claim 11 was conceived of "no later than February 4, 2002," and relies on RI00106817-18 to support this assertion. *Id.* at 4. I disagree with Bio-

Rad's assertions. The cited documents do not demonstrate the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as of the dates alleged.

206. RI00111660–70, which Bio-Rad relies on to evidence conception of claims 1-3, 5, 8-9, and 13, appears to be a portion of a PowerPoint presentation. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 16, 2001. As an initial matter, while the first page of the presentation is dated October 16, 2001, the document's metadata does not indicate when this PowerPoint was last modified or otherwise substantiate this date. Further, the document does not establish that the inventors had possession of every feature recited in claims 1-3, 5, 8-9, and 13, or that every limitation of these claims was known to the inventors as of this date. For example, each claim of the '407 patent requires the formation of a plug, "the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel." This limitation is not suggested by any of the figures in RI00111660–70, which depict channels with regions occupied by "oil" and regions occupied by mixtures of aqueous fluids. The aqueous fluids do not appear to be substantially surrounded by oil. Instead, the aqueous fluid regions depicted appear to be "slugs:"

Solution - true plug flow?



See, e.g., RI00111663. In fact, the slide suggests expanding elements are necessary to achieve stable flow. Unstable flow results when aqueous streams contact the channel, which is indicative of a hydrophilic channel. This would suggest that the aqueous plug fluid can be in contact with the channel wall without significant encapsulation by the carrier oil fluid. Further, the channel layout depicted in these slides is not appropriate for plug formation. Instead of the T-junction approach later used in the Ismagilov patents, these slides depict a single oil channel between two aqueous channels. These channels do not intersect the main channel at an angle. This channel layout is also not appropriate for flow focusing as the two aqueous channels surround a single channel. The inverse would be used for flow focusing (two oil channels surrounding a single

aqueous channel). Based on my experience, this channel layout would not enable plug formation.

207. As another example, each claim of the '407 patent requires “continuously flowing an aqueous fluid” and “continuously flowing a carrier fluid immiscible with the aqueous fluid” to form “at least one plug.” These limitations are not suggested by any of the slides in RI00111660–70, which do not depict or describe the formation of plugs from a “continuously flowing aqueous fluid” and “continuously flowing a carrier fluid immiscible with the aqueous fluid.” Instead, as discussed above, the slides appear to depict “slugs” and the slides do not provide any indication that the aqueous fluid and oil are “continuously flowing.”

208. As another example, claim 2 recites the method according to claim 1 wherein “at least one biological molecule is DNA or RNA,” but this limitation is not suggested by any of the slides in RI00111660-70, which do not depict or describe the formation of plugs containing DNA or RNA. Similarly, for example, claim 13 recites the method according to claim 1, in which one step “includes heating,” but there is no depiction or description of heating in RI00111660-70.

209. As another example, claim 9 recites the method of claim 1 “wherein the oil comprises a surfactant,” but this limitation is not suggested by any of the slides in RI00111660-70, which do not depict or describe any surfactant.

210. RI00111690-738, which Bio-Rad relies on to evidence conception of claim 4, appears to be a grant application that was signed on October 30, 2002. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 30, 2002, or establish that the inventors had possession of every feature recited in claim 4 as of that time. For example, although claim 4 recites “[t]he method according to claim 2, wherein the reaction is a polymerase chain reaction,”

and claim 2 recites “[t]he method according to claim 1, wherein the at least one biological molecule is DNA or RNA,” the only specific reference to DNA or RNA in RI00111690–1738 is the statement that “schemes can be readily constructed in which a single molecule of DNA, RNA, or a protein labeled with nanoparticles is detected visually via [an] autocatalytic pathway” after the molecule is “label[ed]” with metallic autocatalyst—*not* after that molecule undergoes a polymerase chain reaction. RI00111722–23. The only discussion of the “polymerase chain reaction” in this application is as a general example (along with silver halide photography) of a context in which autocatalytic reactions may take place. RI00111719.

211. RI00111308 and RI00111321, which Bio-Rad relies on to evidence conception of claim 10, appear to be entries in a lab notebook. These documents do not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as February 8, 2002, or establish that the inventors had possession of every feature recited in claim 10 as of that time. Bio-Rad has identified no evidence corroborating its apparent assertion that these entries were created on or before February 8, 2002 other than the dates written on these notebook pages (for example, these notes were not witnessed or countersigned by a third party), and one of these entries, RI00111321, bears a date (“3/10/2002”) after February 8, 2002.

212. RI00106817-18, which Bio-Rad relies on to evidence the conception of claim 11, also appears to be two lab notebook entries. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as February 4, 2002. As an initial matter, while the first entry bears the date February 4, 2002, the second entry does not, and Bio-Rad has identified no evidence corroborating its apparent assertion that either of these entries were created on February 4, 2002

(for example, these notes were not witnessed or countersigned by a third party). Further, the document does not establish that the inventors had possession of every feature recited in claim 11, or that every limitation of the claim was known to the inventors as of the date of the entry. For example, claim 11 depends from claim 8, which depends from claim 1, which requires conditions suitable for a “reaction” involving “at least one biological molecule” to be provided in a plug. RI00106817 contains no references to providing conditions for any reaction (whether involving “biological molecules” or otherwise) in plugs. Instead, RI00106817 appears to refer to an experiment where streams of water (with dye) were introduced along with perfluorodecalin into a microchannel, with the observed result that “water and oil plugs were found to form consistently.” The reference to an apparently subsequent experiment involving “Fluorescence testing” in RI00106818 is dated February 5, 2002 and contains no references to plugs, microchannels, or providing conditions suitable for a reaction involving a biological molecule in a microfluidic system.

213. I understand that Bio-Rad asserts that each of claims 1-3, 5, 8-11 and 13 of the was reduced to practice on May 9, 2002, based on the filing of the Ismagilov '927 provisional application on that date. *See* Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 4-5. I further understand that Bio-Rad asserts that claim 4 was reduced to practice on May 9, 2003, based on the filing of U.S. Patent Application No. 10/434,970 (the “'970 application”) on that date. *Id.*

214. While I agree with Bio-Rad's apparent admission that the '927 provisional application fails to contain sufficient written description to establish that the inventors had possession of the alleged invention of claim 4 of the '407 patent, and/or fails to enable claim 4 of the '407 patent, I also believe that the '927 provisional application does not contain sufficient

written description to establish that the inventors had possession of any of the alleged inventions of asserted claims in the '407 patent, and fails to enable any of the asserted claims of the '407 patent. This is discussed in more detail below, for example, because all of the deficiencies identified in the specification of the '407 patent are also present in the '927 provisional application. Accordingly, the listed claims of the '407 patent are also not entitled to claim priority to the '970 application.

215. I understand that Bio-Rad has not provided evidence of the inventors' diligence in reducing the alleged inventions of the '407 patent to practice after Bio-Rad's alleged dates of conception, and consequently that there is no evidence that any claim of the '407 patent would be entitled to priority as of Bio-Rad's alleged dates of conception, even if these dates were uncontested (which they are not). Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 4-5. While Bio-Rad cites a number of documents "*see also*" and "*see, e.g.*" many of these documents do not appear to relate to diligence, and none establish diligence. *Id.* at 5. For example, RI00111541-47, RI00111558-70, and RI00111572-79 are undated. RI00111688-89, RI00111794-97, RI00111580-636, RI00111679-83, RI0011690-738, RI00111673-76, and RI00111739-793 are dated on or after the alleged date of reduction to practice for many of the asserted claims. RI00111677-78 appears to be an email from Heinrich Jaeger to Rustem Ismagilov dated April 24, 2002. The email speaks, in the future tense, of potential future projects that "use microfluidics." It does not describe work which had been or was currently being performed. RI00111684-87 appears to be an email chain between Rustem Ismagilov, Vince Turitto, and Connie Hall dated March 30, 2002 to April 24, 2002. This email chain again speaks of potential future work, not work that had been or was currently being performed. RI00111571 appears to be typed notes titled "Microfluidics Assessment 3/15/2002"

that refer generally to “[f]orming plugs.” While these notes appear to describe work that had been performed relating to “plugs” it is not clear when this work was performed and RI0011571 is dated nearly five months after the alleged conception date for many of the asserted claims. Further, as set forth in **Exhibit 2**, I have reviewed various lab notebooks from Dr. Ismagilov’s lab dated from before May 9, 2003. None of these notebooks suggest that any work was done to reduce the inventions claimed in the ’407 patent to practice in the three months between the alleged date of conception of claims 1-3, 5, 8-9, and 13 (October 16, 2001) and January 16, 2002. For example, none of these lab notebooks include experiments or work relating to conducting reactions in plugs before January 16, 2002. As another example, none of these notebooks suggest that any work was done to reduce the invention claimed in claim 4 of the ’407 patent to practice in the nearly seven months between the alleged date of conception (October 30, 2002) and the alleged date of reduction to practice (May 9, 2003). For example, none of these notebooks include experiments or work relating to conducting PCR in plugs.

216. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception, diligence, or reduction to practice (and I understand that 10X’s position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

B. Invalidity Overview

217. As shown in further detail below, my opinions regarding the ’407 patent include the following:

- All asserted claims are invalid under Section 112 for lack of proper written description, lack of enablement, and/or indefiniteness.
- All asserted claims are anticipated by Quake under Section 102.
- Claims 1, 2, 5, 8, 9, and 13 are anticipated by Shaw Stewart under Section 102.

- All asserted claims are obvious in light of Quake under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Shaw Stewart under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Burns (2001) under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Nisisako under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Thorsen under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Seki under Section 103 (either alone or in combination with other references).

C. Invalidity Under 35 U.S.C. § 112

218. As described in further detail below, it is my opinion that the asserted claims of the '407 patent are invalid under 35 U.S.C. § 112.

1. *Written Description*

219. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '407 patent are invalid for lack of written description.

220. The claims of the '407 patent, for example, require a **“reaction”** with a **“biological molecule.”** I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Bio-Rad appears to be taking the position that “reaction” with a “biological molecule” is far broader than what was disclosed in the '407 patent. Based on Bio-Rad's 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal

cycling protocol.” Infringement of U.S. Patent No. 8,329,407 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1, 11, 36, 61, 63, 79; *see also* Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4,

” *See, e.g.,* Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 8. I have reviewed the ’407 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. The specification describes a single DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’407 patent at 45:30-33; *see also* ’407 patent at 3:52-54. There is no mention in the ’407 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. Indeed, there is no indication that the inventors of the ’407 patent contemplated any DNA amplification reaction beyond the basic (and well-known) PCR reaction. Nor has Bio-Rad identified any disclosure in the ’407 patent specification that discloses other DNA amplification reactions.

221. There is also, for example, no adequate description of performing a “**reaction**” with a “**biological molecule**” in plugs *outside of a substrate*, including, for example, a DNA amplification reaction in plugs outside of a substrate. I have reviewed the ’407 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. There is no indication that the inventors of the ’407 patent contemplated performing a DNA amplification reaction in plugs outside of the substrate. Nor has Bio-Rad identified any disclosure in the ’407 patent specification that discloses a DNA amplification reaction in plugs outside of the substrate.

222. Bio-Rad has taken the following position:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, none of the identified reactions are DNA amplification reactions.

223. Further, in each of the “embodiments in which droplets are captured in a capillary tube” (the “capillary tube embodiments”) identified by Bio-Rad, the droplets remain separated by carrier-fluid such that the risk of droplet coalescence is minimized.

224. First, Bio-Rad cites the description of “a microfluidic device of the present system can further include capillary tubing suitable for collecting plugs (“the capillary device”; FIG. 46). . . . [where] [u]pon formation of plugs in the PDMS portion and their transfer into capillary tubing, the flow rates are stopped, the capillary tubing is disconnected from the PDMS portion and the ends are sealed by capillary wax.” ’407 patent at 59:18-20, 36-39. As shown in Figure 46, this “capillary device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

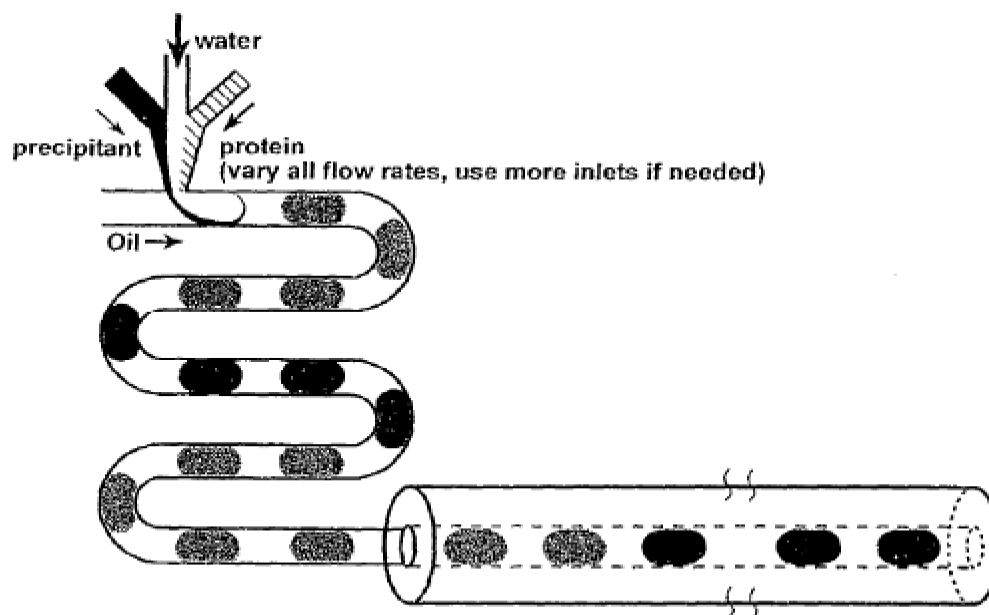


FIGURE 46

225. Second, Bio-Rad cites Example 18 which states: “The capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator (18° C.). A lysozyme crystal appeared within an hour and was stable for at least 14 days without change of size or shape (FIG. 47A).” ’407 patent at 76:30-34. As shown in Figure 47A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

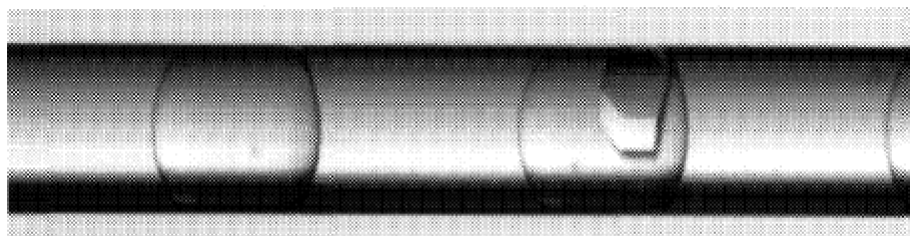


FIGURE 47A

226. Third, Bio-Rad cites Example 19 which states: “The capillary was cut from the PDMS device, sealed by wax and stored in an incubator (18° C.). The thaumatin crystal appeared

in 2-3 days and was stable for at least 45 days without size or shape change (FIG. 47B).” ’407 patent at 76:54-57. As shown in Figure 47B, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

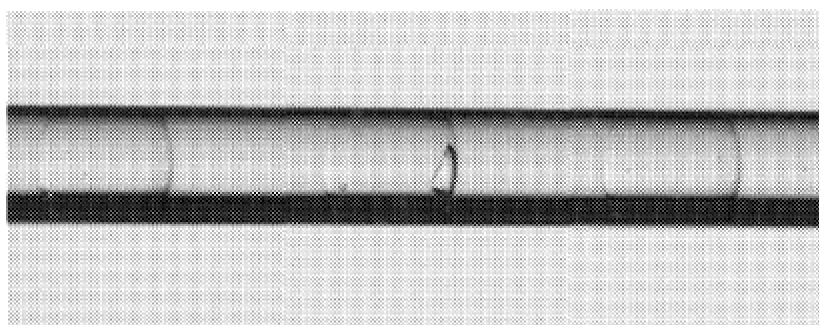


FIGURE 47B

227. Fourth, Bio-Rad cites Example 20 which states, in reference to Figure 50A: “After establishing alternating aqueous droplet streams in the capillary, the flows were stopped, and the capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator at 18° C.” ’407 patent at 77:35-45. As shown in Figure 50A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

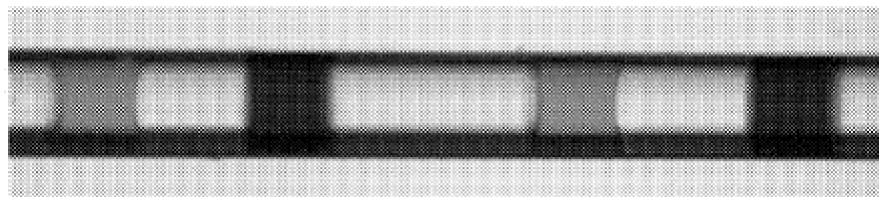


FIGURE 50A

228. The “capillary tube embodiments” generally describe an extension of the microchannel that can be removed from the substrate while maintaining plug separation. These embodiments would not convey to a POSA that the inventors contemplated performing reactions in plugs outside of the substrate, for example in a well as performed by 10X. I understand that

the image below in an image of droplets,

229.

Due to a difference in oil and water density, oil will drain out of the emulsion such that the droplets are closer together, increasing the potential of coalescence. The same is true for droplets collected using a micropipette or centrifuge tube. The capillary tube embodiments would not convey to a POSA that the investors had contemplated collection of droplets under these conditions. Further, as discussed below, Bio-Rad has not identified any teaching in the '407 patent that would convey to a POSA that the inventors had possession of a surfactant that would stabilize droplets and prevent droplet coalescence to allow for a "reaction" with a "biological molecule" in plugs outside of the substrate, let alone a DNA amplification reaction in plugs outside of the substrate.

230. In addition to the "capillary tube embodiments," Bio-Rad cites to a portion of the specification as "*contemplat[ing]* collection of droplets and removal from the chip," First Supplemental Response to 10X's Interrogatory No. 3 (emphasis added):

Thus, devices according to the invention may have a plurality of analysis units that can collect the *solution* from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted

for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'407 patent at 17:60-67 (emphasis added). As an initial matter, this section "contemplates" collecting "*solution*" not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in "a standard 1.5 ml centrifuge tube" or "[c]ollection . . . using micropipettes,"⁹ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection, and subsequent DNA amplification off the substrate.

231. Bio-Rad has taken the position that "the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip" and cites to Figure 24. First Supplemental Response to 10X's Interrogatory No. 3. However, as discussed below, the surfactant depicted in Figure 24 would not stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets outside of the substrate.

232. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927,¹⁰ these applications lack adequate description of performing a "**reaction**" with a "**biological molecule**," including,

⁹ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 ("Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes." The location of reactions is not limited to the substrate. which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes."); *see also* Quake at [0148].

¹⁰ I understand that Bio-Rad is not currently claiming that claim 4 is entitled to claim priority to these applications. Plaintiffs' Corrected Response to Interrogatory No. 1 at 5.

for example, a DNA amplification reaction. I have reviewed the '544 and '927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. The specifications of the '544 and '927 provisional applications do not include a single reference to a DNA amplification reaction. There is no mention in the '544 or '927 provisional applications of (for example) *any* DNA amplification reactions, let alone the details necessary to carry out said reactions. Nor has Bio-Rad identified any disclosure in the '544 or '927 specifications that discloses DNA amplification reactions.

233. The applications also lacks adequate description of performing a “**reaction**” with a “**biological molecule**” outside of a substrate, including, for example, a DNA amplification outside of a substrate. I have reviewed the '544 and '927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the '544 and '927 provisional applications that discloses a DNA amplification reaction in plugs outside of the substrate.

234. Bio-Rad has taken the following position in its Response to 10X's Interrogatory No. 3:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X's Interrogatory No. 3.

235. But the '544 and '927 provisional applications do not describe a single “embodiment in which droplets are captured in a capillary tube,” let alone a DNA amplification reaction outside of the substrate.

236. The specifications of the '544 and '927 provisional applications state:

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'544 application at 28:22-26; '927 provisional application at 27:14-23 (emphasis added). Again, this section speaks to collecting “*solution*” not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in “a standard 1.5 ml centrifuge tube” or “[c]ollection . . . using micropipettes,”¹¹ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection.

237. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X's Interrogatory No. 3. The '544 and '927 provisional applications do not include this figure, or any related discussion. The '544 and '927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” '927 provisional

¹¹ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

application at 12:16-17; '544 application at 12:19-13:5,¹² and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application at 10:31-11:15.¹³ However, as

¹² Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

¹³ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for

discussed below, none of the surfactants described would stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets outside of the substrate.

238. There is also, for example, no adequate description of **“providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.”** I understand the Court has construed “providing conditions suitable for the reaction” to mean “providing a set of physical and chemical conditions that allow the reaction to occur.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Bio-Rad contends that 10X “provid[es] conditions suitable,” which includes “the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction,” by, for example, “plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,329,407 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 61-63; *see also* Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 38. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4, Bio-Rad contends that 10X “provides conditions suitable” which include

the biocompatible conditions within the droplet that allow for enzymes to

example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

function and the appropriate levels of reagents for the DNA amplification reaction (all Chromium Products).” *See e.g.*, Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 41. I have reviewed the ’407 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the ’407 patent that discloses providing conditions suitable for a DNA amplification reaction to occur, let alone for a DNA amplification reaction to occur in plugs outside of the substrate.

239. The specification describes a single DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’407 patent at 45:30-33; *see also* ’407 patent at 3:52-540. The specification does not set forth “a set of physical and chemical conditions that allow [any DNA amplification] reaction to occur.” There is no mention in the ’407 patent of (for example) any DNA amplification reactions other than PCR, let alone “a set of physical and chemical conditions that allow [said] reaction to occur.”

2. *Enablement*

240. As described in further detail above, I have reviewed various documents regarding Bio-Rad’s infringement position in this case. Based on these documents, it is my opinion that the claims of the ’407 patent are invalid for lack of enablement.

241. Claims of the ’407 patent, for example, require a “**reaction**” with a “**biological molecule**.” I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Bio-Rad’s 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,329,407 by 10X’s

GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 11, 36, 61, 63, 79; *see also* Appendix C to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4,

" Appendix C to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 8. But the specification of the '407 patent does not enable the full scope of the limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. The claims purport to cover *all* DNA amplification reactions in plugs (whether known or unknown at the time of Ismagilov's alleged invention), but the specification describes a single DNA amplification reaction: "Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences." '407 patent at 45:30-33; *see also* '407 patent at 3:52-54. The specification does not include a working example of a PCR reaction in plugs. And there is no mention in the '407 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. The '407 patent fails to disclose, teach, or suggest how to conduct every "DNA amplification reaction," and particularly, the "DNA amplification reactions" allegedly performed by 10X,¹⁴ within plugs.

I understand that these techniques were developed by 10X years after the priority date of the Ismagilov patents.

242. As another example, claims of the '407 patent, for example, require a "**reaction**" with a "**biological molecule**." I understand the Court has construed "reaction" as: "Physical,

¹⁴ I have not been asked to provide, and have not formed an opinion on whether or not the reactions performed in 10X's products are "DNA amplification reactions."

chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Bio-Rad’s 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,329,407 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1, 11, 36, 61, 63, 79; *see also* Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4,

Appendix C to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 8. The claims purport to cover *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov’s alleged inventions), including DNA amplification reactions in plugs *outside of the substrate*. But the specification of the ’407 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. The specification does not enable DNA amplification reactions in plugs *outside of the substrate*. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. Surfactants that would enable a POSA to conduct biological reactions in plugs outside of the substrate, let alone DNA amplification reactions in plugs outside of the substrate, are not described in specification of the ’407 patent and were not even available as of the alleged priority date of the ’407 patent. In fact, surfactants appropriate for this use were not developed or described until 2008—seven years after Ismagilov’s alleged invention.

243. As discussed above, Bio-Rad contends that 10X performs a “DNA amplification

reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.”

244. The surfactants disclosed in the Ismagilov patents would not stabilize droplets under these conditions.

245. In order to conduct biological assays within microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, preventing coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Holtze, C., et al., “Biocompatible surfactants for water-in-fluorocarbon emulsions,” *Lab Chip*, 8:1632-

1639 (2008) (“Holtze”) (10X-000013467-75) at 1632.¹⁵ “These requirements [were] particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluortelomer-tails (typically perfluorinated C₆ to C₁₀) . . . do not provide sufficient long-term emulsion stability.” *Id.*¹⁶ Even as of 2008, years after the priority date of the ’407 patent, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.*

246. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. But none of the surfactants disclosed in the specification of the ’407 patent, including the surfactants disclosed in Figure 24, meet the requirements set forth above.

247. As set forth in the specification, “FIG. 24 shows a reaction scheme that depicts examples of fluorinated surfactants that form monolayers that are: (a) resistant to protein adsorption; (b) positively charged; and (c) negatively charged. Fig. 24b shows a chemical structure of neutral surfactants charged by interactions with water by protonation of an amine or guanidinium group. FIG 24c shows a chemical structure of neutral surfactants charged by interactions with water deprotonation of a carboxylic acid group.” ’407 patent at 5:41-49.

¹⁵ Holtze was authored by individuals from Harvard University, Universit`a del Salento, Lecce, Italy, and Raindance Technologies, Inc. Holtze at 1632.

¹⁶ When conducting biological assays in droplets, “it is attractive to use a fluorocarbon oil as the continuous phase” and accordingly, a fluorosurfactant to “ensur[e] that drops are stable.” Holtze at 1632.

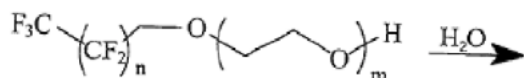


FIG. 24A

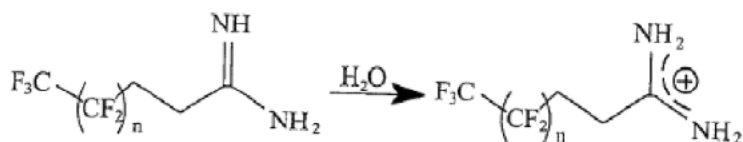


FIG. 24B

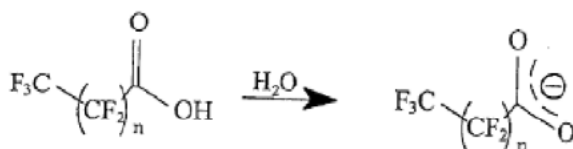


FIG. 24C

Fig. 24

248. Specifically, Figure 24a “depicts a “fluorinated surfactants containing perfluoroalkyl chains [(red)] and an oligoethylene glycol head group [(blue)].” ’407 patent at 75:8-10.

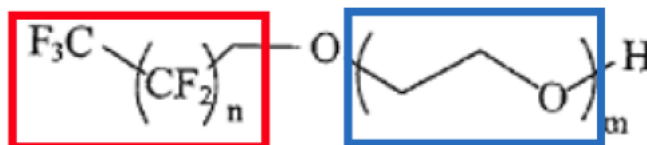


FIG. 24A

249. The surfactant depicted in Figure 24a is commercially available under the trade name Zonyl.” See ’407 patent at 21:21-28 (“Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)”); ’407 patent at 76:64-66 (“A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283.”).

250. Figure 18, depicts the same fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group. ’407 patent at 57:48-50 (“In FIG. 18, plugs are

formed in the presence of several solutions of surfactants that possess different functional groups (left side of the diagram)”) (annotation added).

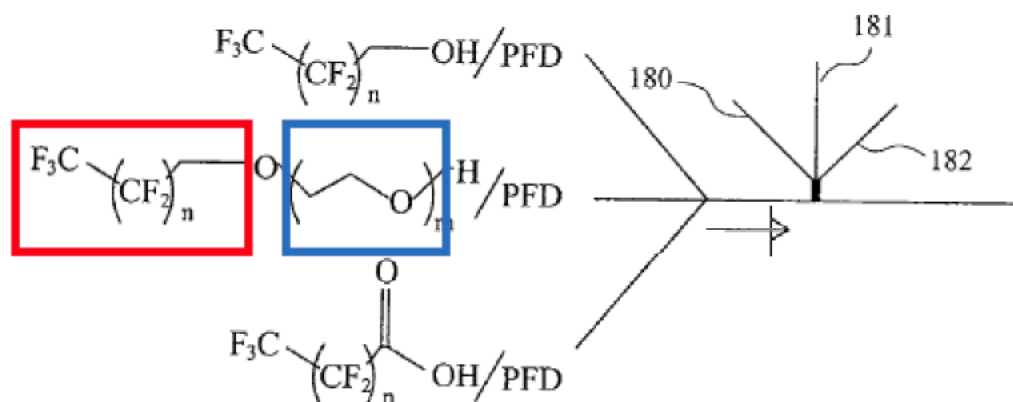


Fig. 18

251. Unlike the ionic surfactants depicted in Figure 24b and 24c, the surfactant depicted in Figure 24a meets the second requirement set forth above. It will “produce a biologically inert interior surface for the water drops.” As described in the specification of the ’407 patent: “[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.” ’407 patent at 36:35-39. Further, this OEG head group is non-ionic as required for biological assays. Holtze at 1632. But this surfactant does not meet the first requirement set forth above for performing biological assays in droplets. Specifically, it would not “provide stability to the drops, preventing coalescence.”

252. The surfactant depicted in Figure 24a contains “a “perfluoroalkyl chain[] and an oligoethylene glycol head group.” ’407 patent at 75:8-10. A perfluoroalkyl chain (also referred to as a “perfluoroalkyl tail” of “fluorotelomer-tail”) is not sufficient to stabilize droplets outside of the substrate. As described by Holtze *et al.* “[s]urfactants with short fluorotelomer-tails” like the

perfluoroalkyl chain depicted in Figure 24a, “do not provide sufficient long-term emulsion stability.” Holtze at 1632.

253. I understand that Dr. Jeremy Agresti, Bio-Rad’s R&D Director and a co-author on Holtze et al., confirmed this point. Dr. Agresti was questioned regarding the text copied below from Holtze et al.:

However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C₆ to C₁₀) have been used, but do not provide sufficient long-term emulsion stability. Fluorosurfactants with longer fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632 (internal citations omitted).

254. Dr. Agresti confirmed that these statements were accurate at the time they were written in 2008. Agresti Tr. 199:9-13 (“Q. Do you believe that the statements that are made in

the article that you just read, those portions that the article that you just read, do you believe those are accurate? A. Yeah, at the time for sure.”).

255. Further in reference to the following statement in Holtze et al.: “Biological assays thus demand fluorosurfactants with non-ionic head groups; however, there are currently no such surfactants available,” Dr. Agresti confirmed that as of 2008 there were no “flourosurfactants with nonionic head groups that would stabilize and emulsion long term.” Agresti Tr. 202:2-13 (“Q. And it was true that as of – as of the date of this article, which was 2008, that at least to your knowledge that there were no nonionic fluorosurfactants with nonionic head groups? A. That could stabilize an emulsion long term. We knew that there were fluoro surfactants with nonionic head groups. Q. [W]hat was not known was that there were fluoro surfactants with nonionic head groups that would stabilize an emulsion long term. A. Yes, that’s right.”). Dr. Agresti further confirmed that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, specifically Zonyl, “doesn’t stabilize droplets for PCR.” Agresti Tr. at 203:10-19 (Q. Are you familiar with a surfactant known as . . . ZONYL? A. Yes. Q. Has Bio-Rad used that surfactant? A. I can’t say. It’s not in any product. As far as I know it’s never been in any product. Q. Why not? A. As far as I know it doesn’t stabilize droplets for PCR.”).

256. I understand that named inventor of the ’083 patent Mr. Lewis Spencer Roach, who testified that his “primary contribution” was to developing “fluorinated surfactant[s] [with] hydrophilic head group,” Roach Tr. 26:21-23, also confirmed the point that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl would, not stabilize droplets long term. Mr. Roach testified that “other groups have done a lot of work on preventing coalescence using surfactants” but “I did not personally perform that research.” Roach Tr. 78:15-20. When asked whether “other groups” mean “other people in Dr. Ismagilov’s lab,” Mr. Roach

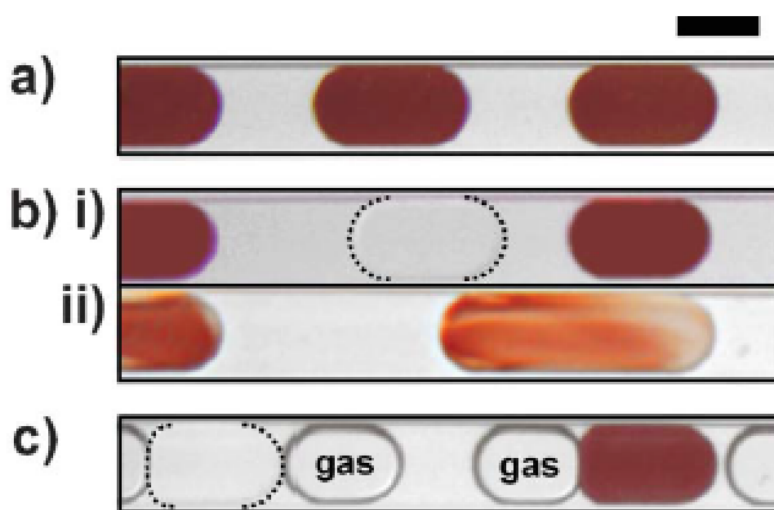
answered that he “believe[d] it was outside of Ismagilov’s group.” Roach Tr. 78:21-79:2. Mr. Roach further testified that the “Rf-OEG surfactant is not optimized for preventing coalescence . . . [t]here are other hydrophilic head groups that are better at controlling adsorption than a simple oligo (ethylene glycol) head group. I think other people have made these.” Roach Tr. at 79:3-12.¹⁷ Mr. Roach later confirmed that the “other people” he was referring to were Holtze et al. in 2008. Roach Tr. 80:4-11 (“A. I believe [Exhibit 129 (Holtze et al.)] is what I was just referring to, that other groups had optimized surfactants to – give me just a second. I want to read the conclusions in this paper here. Q. Certainly. A. Yes. This is where I was discussing other groups that have optimized surfactants to prevent coalescence or merging of plugs.”).

257.

¹⁷ The “Rf-OEG” surfactant is a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group. *See* Roach Depo. Ex. 127 (“Perfluorinated-tail, oligoethylene glycol derivatized molecules (Rf-OEG) were selected as a neutral and hypothetically biocompatible surfactants.”); Roach Tr. 51:22-23 (“A. I synthesized the surfactants described in [Exhibit 127], particularly the Rf-OEG surfactant.”).

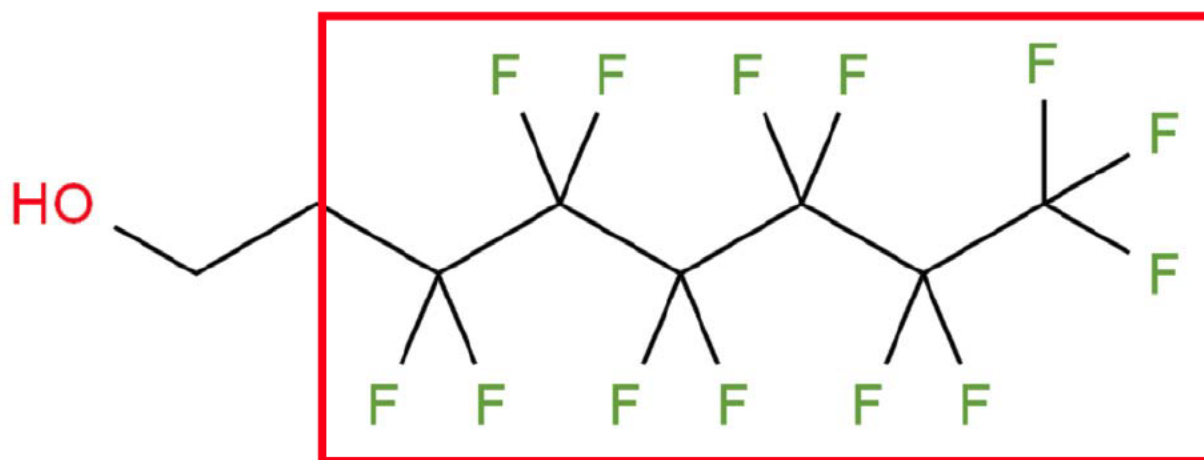
258. I agree with Agresti's, Roach's, and Hindson's statements above regarding fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl. Such surfactants would not provide stability to drops and prevent coalescence to allow for DNA amplification reactions in microfluidic droplets outside of the substrate.

259. Dr. Ismagilov himself recognized the potential for coalescence, even between plugs within the substrate. As explained by Dr. Ismagilov, "[d]uring flow, plugs with different chemical composition may move relative to the carrier fluid at different rates and thus move relative to one another allowing adjacent plugs to coalesce (Fig. 2(b))." Adamson, D., et al., "Production of arrays of chemically distinct nanolitre plugs via repeated splitting in microfluidic devices," *Lab Chip*, 6:1178-1186 (2006) ("Adamson") (10X-000254852-70) at 1181.



260. Figure 2b above depicts coalescence between "[p]lugs of distinct chemical

composition.” Adamson at 1181. The carrier fluid is “FC-3283 10:1 PFO (v/v) throughout.” Adamson at 1181. FC-3283 is a fluorinated oil. PFO or 1H,1H,2H,2H-perfluorooctanol is a fluorinated surfactant. The chemical formulation of PFO is depicted below:

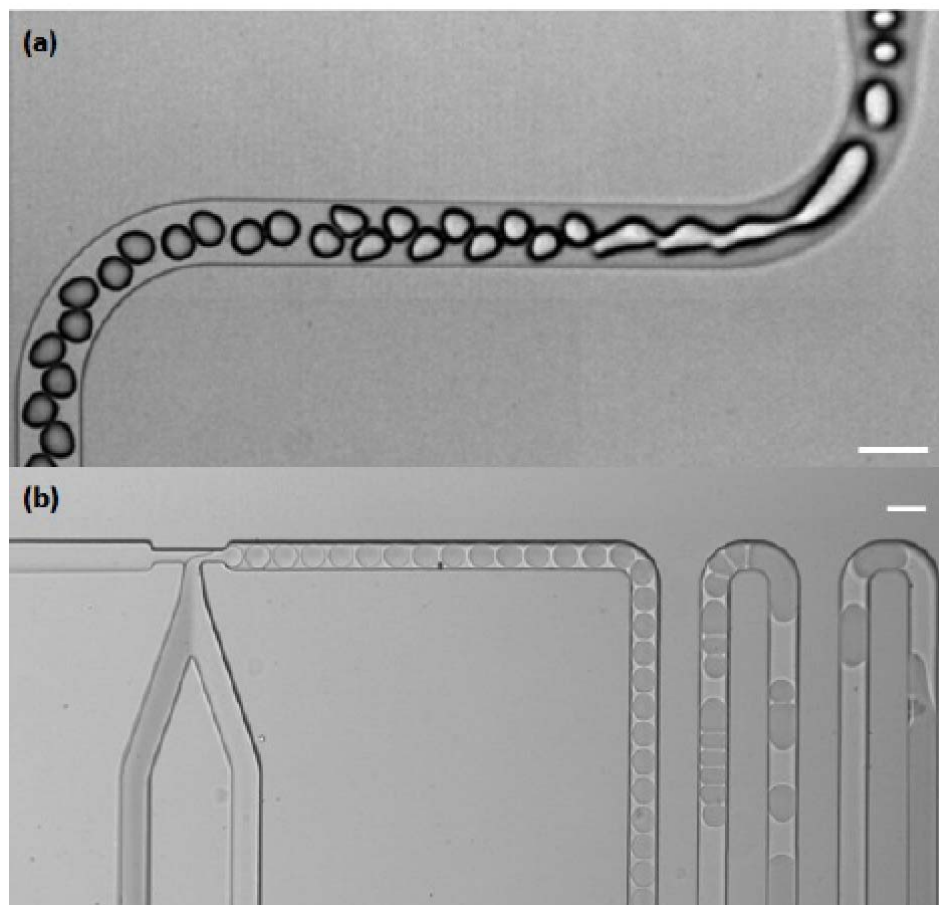


261. Like Zonyl, PFO contains a short perfluoroalkyl tail (red).

262. As explained by Dr. Ismagilov, this surfactant was insufficient to prevent coalescence even within the substrate. *See* Adamson at 1181. Instead “[t]o prevent coalescence, gas bubbles [were] introduced as spacers between plugs to (1) minimize the relative motion of plugs and (2) to act as a physical barrier to prevent the coalescence of adjacent plugs during flow and splitting.” Adamson at 1181.

263. This potential for droplet coalescence was later described as “[u]ncontrolled.” Cho, S.D., “An integrated droplet based microfluidic platform for high throughput, multi-parameter screening of photosensitiser activity,” Doctoral Thesis, Department of Chemistry, Imperial College London (2013) (“Cho Thesis”) (10X-000254992-5215) at Fig. 3.1. This thesis explained: “Perfluorodecalin and 1H,1H,2H,2H-perfluoro-1-octanol combination was used for studying protein crystallization by Ismagilov and his coworkers. The perfluorinated oil and surfactants are advantageous for microdroplet based biochemical applications as they are

lipophobic, inert, insoluble in water and compatible with many biochemical molecules. Unfortunately, droplets in perfluorodecalin oil with 1H,1H,2H,2H-perfluoro-1-octanol were not stable and merged with each other under pressure (Figure 3.1(b)[I]).” Cho Thesis at 51. The “[d]roplet generation and uncontrolled coalescence of droplets in perfluorodecalin with 5% v/v 1H, 1H, 2H, 2H-perfluoro-1-octanol” observed is depicted in (b) below:



264. “Fluorosurfactants with longer fluorocarbon tails” are required for “long-term stabilization” to perform biological assays. Holtze at 1632. As further described by Holtze et al., as of 2008, no such surfactant existed in 2008:

However, the only available PFPE-based surfactants have ionic headgroups, e.g. poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as

DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632. Holtze et al. disclose examples of fluorinated surfactants meeting the two requirements set forth above. These surfactants comprise non-ionic polyethylene glycol head groups and perfluorinated polyether tails. Holtze at 1; Figure 2.

265.

266. I understand that RainDance's droplet products utilize a "biocompatible surfactant, PEG-PFPE block copolymer." Plaintiffs' First and Second Supplemental Response to Interrogatory No. 5. Bio-Rad's droplet products utilize "Krytox K225 (0.58mM) + perfluorodecanol (0.625 mM) or the BRDG3 triblock fluorosurfactant." Plaintiffs' Third and Fourth Supplemental Response to Interrogatory No. 5.

267. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application

60/394,544 or U.S. Provisional Application No. 60/379,927, the specifications of the '544 and '927 provisional applications do not enable a person of skill in the art to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate without undue experimentation. Surfactants necessary to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate are not described in specification of the '544 or '927 provisional applications and were not even available as of the filing date of the '544 or '927 provisional applications.

268. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The '544 and '927 provisional applications do not include this figure, or any related discussion. The '544 and '927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” '927 provisional application at 12:16-17; '544 application at 12:11-13,¹⁸ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic

¹⁸ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application, 10:31-11:15.¹⁹ In the context of conducting biological assays in microfluidic droplets outside of the substrate, each of the surfactants listed—excluding “fluorinated oil” discussed separately in below—would be considered an aqueous soluble surfactant by a POSA, meaning they are introduced in the aqueous phase instead of the oil phase. To conduct biological assays in microfluidic droplets outside of a substrate, a POSA would understand that a continuous phase comprised of a fluorinated oil is preferred, if not necessary. Holtze at 1632. The listed surfactants are non-

¹⁹ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopahnitrate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

fluorinated and as such are not soluble in fluorinated oil. Therefore, to use one of the listed surfactants in a system comprising a fluorinated oil, the surfactant needs to be introduced into the aqueous phase. However, when present in the aqueous phase these surfactants would be disruptive to emulsion stability. The hydrophobic portions of these surfactant molecules cause them to populate the aqueous-fluorinated oil droplet boundary, displacing any stabilizing fluorinated surfactant molecules present. This process leads to droplet coalescence rather than stabilization.

269. “Fluorinated oil” while soluble in fluorinated oil, also would not stabilize droplets to conduct biological assays in microfluidic droplets outside of the substrate. Holtze at 1632 (“[I]t is attractive to use a fluorocarbon oil as the continuous phase However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable.”).

270. As another example, claims of the ’407 patent require **“providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.”** I understand the Court has construed “providing conditions suitable for the reaction” to mean “providing a set of physical and chemical conditions that allow the reaction to occur.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Bio-Rad contends that 10X “provid[es] conditions suitable,” which includes “the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction,” by, for example, “plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,329,407 by 10X’s GemCode Platform

(Plaintiffs' 4(c) disclosures) at 61-63; *see also* Appendix C to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 38. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X "provides conditions suitable" which include

the biocompatible conditions within the droplet that allow for enzymes to function and the appropriate levels of reagents for the DNA amplification reaction (all Chromium Products)." Appendix C to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 41. The claims purport to cover providing conditions suitable for *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov's alleged inventions), including conditions suitable for DNA amplification reactions in plugs *outside of the substrate*. But the specification of the '407 patent does not enable the full scope of this limitation, at least under Bio Rad's actual and/or apparent application of the claims, without undue experimentation. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. The '407 patent fails to disclose, teach, or suggest how to provide all conditions suitable for *any* reaction between a biological molecule and a reagent in plugs outside of the substrate, including all the conditions suitable for a DNA amplification reaction in plugs outside of the substrate.

271. Bio-Rad has taken the position that "the patents-in-suit disclose a comprehensive toolkit for conducting reactions in chemical droplets." First Supplemental Response to 10X's Interrogatory No. 3. Specifically, Bio-Rad has taken the position that "the patents-in-suit teach precisely: (1) the types of fluorinated oils and surfactants that have been used throughout the industry for this purpose; (2) the types of microfluidic devices that have been used for this purpose; and (3) the ability to precisely control the composition of droplets so that DNA

amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, Bio-Rad provides no explanation for its assertions. As explained above, “the types of fluorinated oils or surfactants” described in the ’407 patent have *not* “been used throughout the industry for this purpose.” Instead, as explained above, each of 10X, RainDance, and Bio-Rad utilize surfactants with a non-ionic polyethylene glycol head group and a perfluorinated polyether tail, or tails. Further, the ’407 specification does not teach “the ability to precisely control the composition of droplets so that DNA amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. The ’407 patent does not describe the “composition of droplets” necessary to conduct *any* DNA amplification.

3. *Indefiniteness*

272. It is my opinion that the claims of the ’407 patent are invalid as indefinite because the ’407 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention.

273. For example, each claim of the ’407 patent purport to cover “**providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**” I understand the Court has construed “providing conditions suitable for the reaction” to mean “providing a set of physical and chemical conditions that allow the reaction to occur.” Claim Construction Order at 1. But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “a set of physical and chemical conditions that allow the reaction to occur.” For example, the specification does not inform, with reasonable certainty which “set of physical and chemical conditions” would allow a DNA amplification reaction to occur. Without an adequate description of what constitutes “conditions suitable” for the reaction between the biological molecule and the reagent, a POSA could not know whether he or she was practicing the claims.

274. As another example, each claim of the '407 patent purports to cover a plug being “substantially surrounded by the immiscible carrier fluid flowing through the channel.” But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “**the channel**.” Independent claim 1 of the '407 patent references “a first channel” and a “second channel”:

A method for conducting a reaction in plugs in a microfluidic system, comprising the steps of:

providing the microfluidic system comprising *at least two channels* having at least one junction;

continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through *a first channel* of the at least two channels;

continuously flowing a carrier fluid immiscible with the aqueous fluid through *the second channel* of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the *at least two channels*, the plug being substantially surrounded by the immiscible carrier fluid flowing through *the channel*

275. The claim does not specify whether “the channel” is the “first channel” or the “second channel.”

D. Invalidity Based on Prior Art

1. Anticipation

(a) Invalidity Based on Quake

276. It is my opinion that Quake discloses all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

277. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

278. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

279. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

280. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

281. Quake even describes a specific type of chemical reaction involving enzymes

produced by cells:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

282. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

283. Quake satisfies this limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (*e.g.*, molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

284. Quake discloses that the microfluidic devices described contain at least two channels having at least one junction. For example, Quake states that “[t]he devices and methods of the invention comprise *a main channel*, through which a pressurized stream of oil is passed, and *at least one sample inlet channel*, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion’ region joins the sample inlet channel to the main*

channel such that the aqueous solution can be introduced to the main channel, e.g., at an angle that is perpendicular to the stream of oil.” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0068] (“The main channel is typically in fluid communication with an inlet channel or inlet region, which permits the flow of molecules, cells or virions into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a *junction between an inlet region and the main channel of a chip of the invention*”).

285. Figure 16A in Quake also illustrates this limitation. Figure 16A is reproduced below:

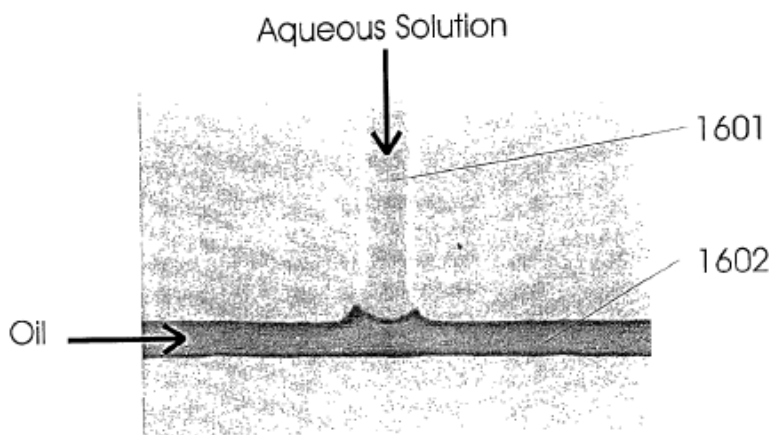


FIG. 16A

286. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

287. Quake satisfies this limitation. For example, Quake describes the devices and

methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, *through which a pressurized stream of aqueous solution is passed.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

288. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device *and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.* The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample.*” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the *biological material may be, e.g., molecules* (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

289. Quake also made clear that the “flow” of the aqueous fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the

embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining *a flowing stream of an aqueous solution* and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

290. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

291. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

292. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid,*

which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

293. Quake describes that the “force and direction” of the flow of carrier fluid “can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels).” Quake at [0125].

294. Quake also made clear that the “flow” of the carrier fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and *a flowing stream of an immiscible fluid (e.g., decane)* it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

295. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.**”

296. Quake satisfies this limitation. For example, Quake describes the devices and

methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel*, e.g., at an angle that is perpendicular to the stream of oil. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that *the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream thereby forming droplets.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”).

297. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” Quake at [0020] (emphasis added).

298. Quake also made clear that his patent application described the forming of droplets by partitioning aqueous fluid with carrier fluid. For example, during prosecution of his patent application, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid

(e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15.

299. Claim 1 further recites: “**the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.**”

300. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

301. Claim 1 further recites: “**wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.**”

302. Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

303. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS

may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

304. Quake even describes performing a specific type of reaction involving a biological molecule inside a droplet:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (e.g. fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

305. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

306. Quake satisfies this limitation. For example, Quake discloses the conditions required for the enzymatic reaction described above:

In another embodiment, cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example,

cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170].

(ii) *Claim 2*

307. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

308. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

309. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (*i.e.*, flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample.*” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, *e.g.*, *molecules (for example, polynucleotides*, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added). Quake defines “polynucleotide” as including “*double and single stranded RNA and DNA.*” Quake at [0052] (emphasis added).

310. Quake also discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR.*” Quake at [0080] (emphasis added). A person of ordinary skill in the art would have known that PCR requires sample DNA as a reagent. Mullis, K., et al., “Process for Amplifying, Detecting, and/or-Cloning Nucleic Acid Sequences,” U.S. Patent No. 4,683,195 (filed on February 7, 1986; issued on July

28, 1987) (“Mullis”) (10X-000255404-39) at Abstract, 2:63-3:1.

(iii) *Claim 3*

311. Claim 3 of the ’407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

312. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

313. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the ’407 patent explains, PCR is a type of autocatalytic reaction. *See* ’407 patent at 45:30-33 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

(iv) *Claim 4*

314. Claim 4 of the ’407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

315. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

316. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

(v) *Claim 5*

317. Claim 5 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

318. Claim 5 further recites: “**the reaction is an enzymatic reaction.**”

319. Quake satisfies this limitation. For example, Quake discloses that “[i]n various embodiments of the method, the biological material may be, e.g., molecules (for example,

polynucleotides, polypeptides, *enzymes*, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

320. Quake also describes that the reaction that takes place within a droplet may be an enzymatic reaction. In describing the detection of the results of a reaction, Quake discloses that “[a]s each droplet passes into the detection region, it is examined for a predetermined characteristic (i.e., using the detector) and a corresponding signal is produced . . . the amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the strength of the signal may indicate the size of a molecule, or *the potency or amount of an enzyme expressed by a cell*, or a positive or negative reaction such as binding or hybridization of one molecule to another, *or a chemical reaction of a substrate catalyzed by an enzyme*.” Quake at [0078] (emphasis added); *see also* Quake at [0106] (emphasis added) (“Enzymes can be analyzed and/or sorted by the extent to which they catalyze chemical reaction of a substrate (conversely, substrate can be analyzed and/or sorted by the level of chemical reactivity catalyzed by an enzyme).”).

321. Quake described a particular enzymatic reaction to detect for the presence of a particular enzyme in sample cells. For example, Quake disclosed:

In another embodiment, cells may produce a reporter *in vivo* (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, *cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene)* with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability

of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

322. Quake even provided “an exemplary channel design for compartmentalization of Enzyme and Substrate.” Quake at [0045] (referring to Fig. 22). Figure 22 is reproduced below:

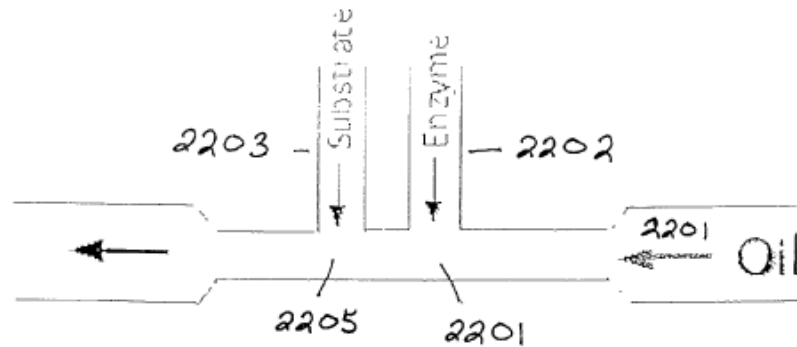


FIG. 22

(vi) Claim 8

323. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

324. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

325. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within *microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0014] (“In a preferred embodiment, water droplets are extruded into a flow of oil”); Quake at [0015] (“For example, the

first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).”); Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane or hexadecane)”; Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane)”).

326. Quake also explains that the carrier fluid, or “extrusion fluid,” is “incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel.” Quake at [0022].

327. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

(vii) *Claim 9*

328. Claim 9 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

329. Claim 9 further recites: “**the oil comprises a surfactant.**”

330. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

331. Quake also states that the carrier fluid, or “extrusion fluid,” may contain

surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

332. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating which minimizes adhesion Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

333. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(viii) *Claim 10*

334. Claim 10 of the '407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

335. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

336. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

(ix) *Claim 11*

337. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

338. Claim 11 further recites: “**the oil is a fluorinated oil.**”

339. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*”).

340. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil

that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

(x) *Claim 13*

341. Claim 13 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

342. Claim 13 further recites: “**the providing step includes heating.**”

343. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Quake at [0080]. A person of skill in the art would have known that the PCR reaction required cycles of heating and cooling. Mullis at 9:55-60.

(b) Invalidity Based on Shaw Stewart

344. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the ’407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

345. The preamble of claim 1 of the ’407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

346. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the

preamble is not limiting.

347. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microlitre” Shaw Stewart at 1:20-22 (emphasis added).

348. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

349. Shaw Stewart satisfies this claim limitation. For example, Shaw Stewart described that a microfluidic system with at least two channels comprising at least one junction, as shown in Figure 1, reproduced below:

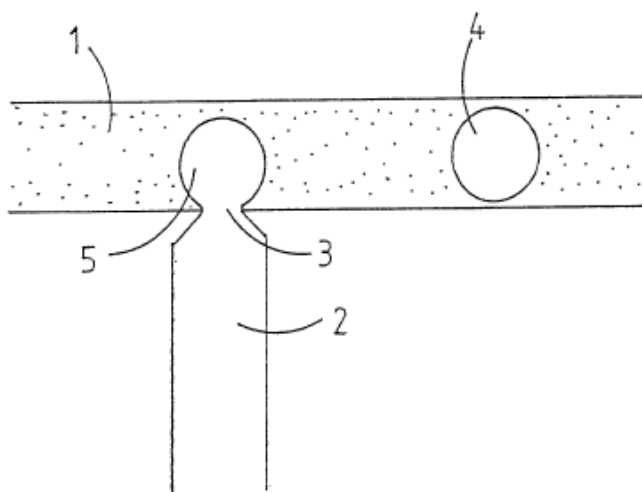


Figure 1.

Shaw Stewart at Fig. 1. In describing the figure, Shaw Stewart stated that “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed

through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

350. Figure 2 of Shaw Stewart, reproduced below, also discloses this limitation.

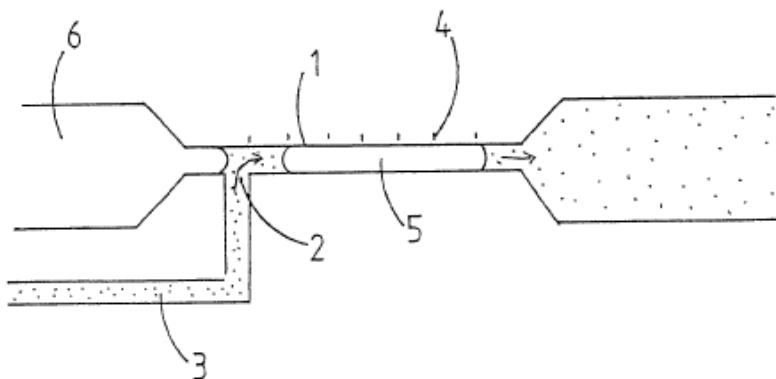


Figure 2.

Shaw Stewart at Fig. 2. In describing Figure 2, Shaw Stewart stated that “[i]n this case the reagent (6) is passed into a tube of considerably narrower bore (1) than the cross-section of the droplets to be produced. The tube is graduated relative to the opening (2) of a side arm (3). The reagent is passed into the tube to a certain graduation (4), whereupon carrier phase is introduced from the side-arm, thus breaking off a droplet to the required size.” Shaw Stewart at 1:96-104.

351. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

352. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each

droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

353. Figure 1 of Shaw Stewart also discloses this limitation.

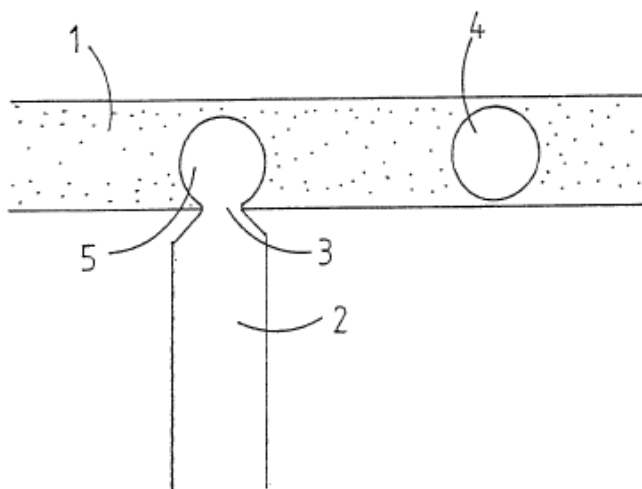


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

354. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution

and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

355. Claim 1 further recites: “continuously flowing a carrier fluid immiscible **with the aqueous fluid through the second channel of the at least two channels.**”

356. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent

liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

357. Figure 1 of Shaw Stewart also discloses this limitation.

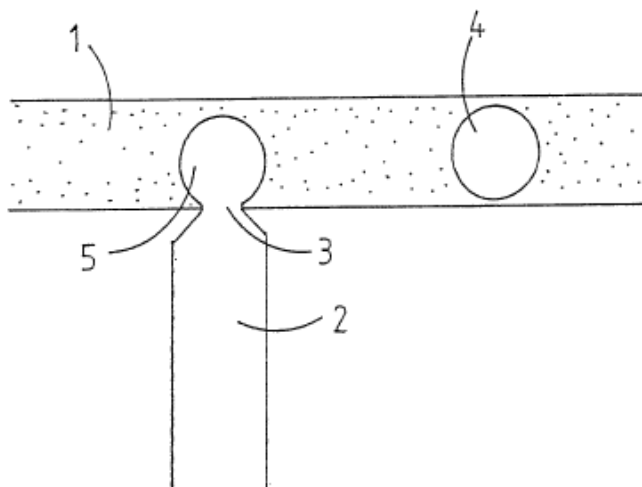


Figure 1.

358. Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

359. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the

Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

360. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.”**

361. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

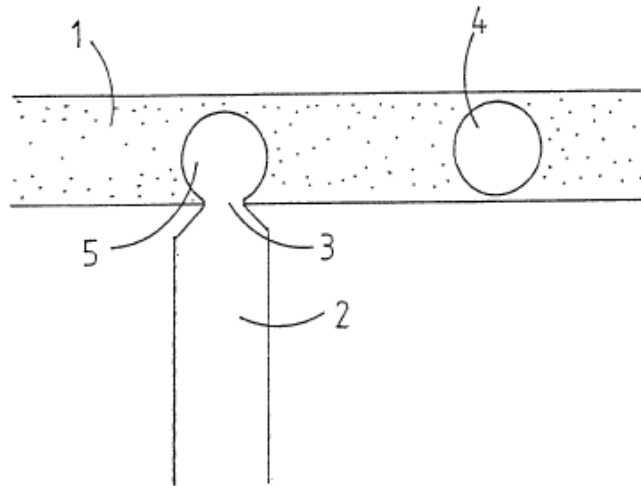


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

362. Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

363. Claim 1 further recites: “**the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.**”

364. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

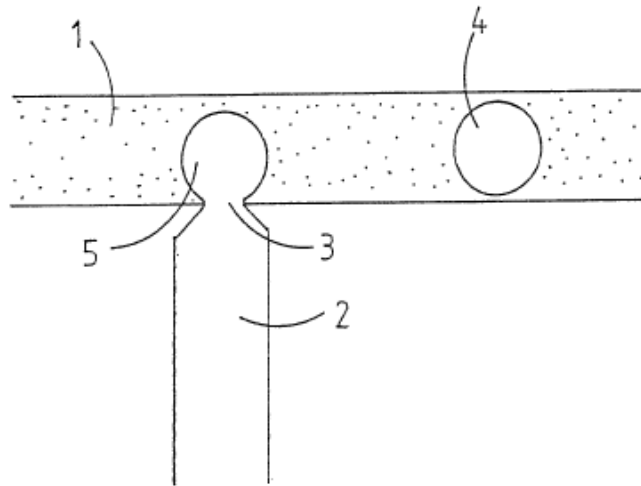


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

365. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

366. Claim 1 further recites: “**wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.**”

367. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as *forensic and recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added). Because Shaw Stewart disclosed that his invention could be applied to, for example, biochemistry and recombinant

DNA work, the droplets formed in Shaw Stewart would have been comprised at least one biological molecule and at least one reagent for conducting the reaction with the at least biological molecule.

368. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

369. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would allow different types of reactions to take place within droplets. Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature*.” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

(ii) Claim 2

370. Claim 2 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

371. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

372. Shaw Stewart satisfies this limitation. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*,

geology, etc., especially in procedures which utilize very small quantities, such as forensic and ***recombinant DNA work***.” Shaw Stewart at 3:82-86 (emphasis added). Because Shaw Stewart disclosed that his invention could be applied to, for example, recombinant DNA work, the droplets formed in Shaw Stewart would have been comprised at least one DNA molecule.

(iii) *Claim 5*

373. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

374. Claim 5 further recites: “**the reaction is an enzymatic reaction.**”

375. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. In order for the inventions described in Shaw Stewart to be applied to “recombinant DNA work,” enzymatic reactions must take place with Shaw Stewart’s droplets. *See generally*, Loenen, M., et al., “Highlights of the DNA cutters: a short history of the restriction enzymes,” *Nucleic Acid Research*, 42:3-19 (2014) (“Loenen”) (10X-000255387-403).

(iv) *Claim 8*

376. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

377. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

378. Shaw Stewart satisfies this limitation. For example, Shaw Stewart states that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

(v) *Claim 9*

379. Claim 9 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

380. Claim 9 further recites: “**the oil comprises a surfactant.**”

381. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioyl, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(vi) *Claim 13*

382. Claim 13 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

383. Claim 13 further recites: “**the providing step includes heating.**”

384. Shaw Stewart satisfies this limitation. For example, Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled

heating coil.”).

2. *Obviousness*

(a) Invalidity Based on Quake

385. It is my opinion that Quake discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

386. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

387. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

388. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

389. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

390. Quake even describes a specific type of chemical reaction involving enzymes

produced by cells:

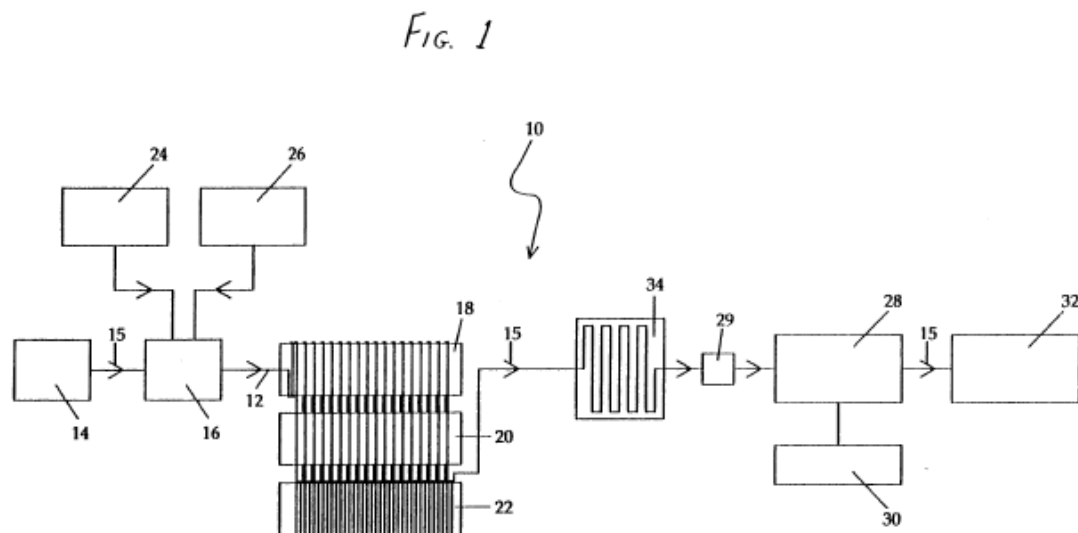
In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

391. While it is my opinion that Quake discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide

triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

392. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose

(HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

393. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

394. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30)

together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

395. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

396. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

397. Quake satisfies this limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

398. Quake discloses that the microfluidic devices described contain at least two channels having at least one junction. For example, Quake states that “[t]he devices and methods of the invention comprise *a main channel*, through which a pressurized stream of oil is passed, and *at least one sample inlet channel*, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion’ region joins the sample inlet channel to the main*

channel such that the aqueous solution can be introduced to the main channel, e.g., at an angle that is perpendicular to the stream of oil.” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0068] (“The main channel is typically in fluid communication with an inlet channel or inlet region, which permits the flow of molecules, cells or virions into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a *junction between an inlet region and the main channel of a chip of the invention*”).

399. Figure 16A in Quake also illustrates this limitation. Figure 16A is reproduced below:

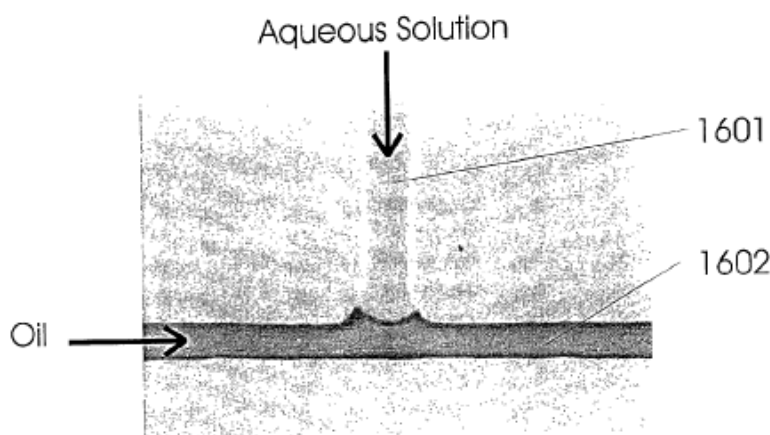


FIG. 16A

400. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

401. Quake satisfies this limitation. For example, Quake describes the devices and

methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, *through which a pressurized stream of aqueous solution is passed.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

402. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device *and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.* The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample.*” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the *biological material may be, e.g., molecules* (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

403. Quake also made clear that the “flow” of the aqueous fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the

embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining *a flowing stream of an aqueous solution* and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

404. While it is my opinion that Quake discloses flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

405. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For

example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

406. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

407. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer

size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

408. It also would have been obvious to flow aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

409. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

410. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the

main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

411. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

412. Quake describes that the “force and direction” of the flow of carrier fluid “can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels).” Quake at [0125].

413. Quake also made clear that the “flow” of the carrier fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and *a flowing stream of an immiscible fluid (e.g., decane)* it is possible to produce

small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.”
Quake ’103 Response at 15 (emphasis added).

414. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.**”

415. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel*, e.g., at an angle that is perpendicular to the stream of oil. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that *the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream thereby forming droplets.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”).

416. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or

flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” Quake at [0020] (emphasis added).

417. Quake also made clear that his patent application described the forming of droplets by partitioning aqueous fluid with carrier fluid. For example, during prosecution of his patent application, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15.

418. While it is my opinion that Quake discloses forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

419. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single

DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

420. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

421. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution

and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

422. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

423. Claim 1 further recites: **“the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.”**

424. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

425. Claim 1 further recites: **“wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.”**

426. Quake satisfies this claim limitation. For example, the abstract in Quake describes

the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

427. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

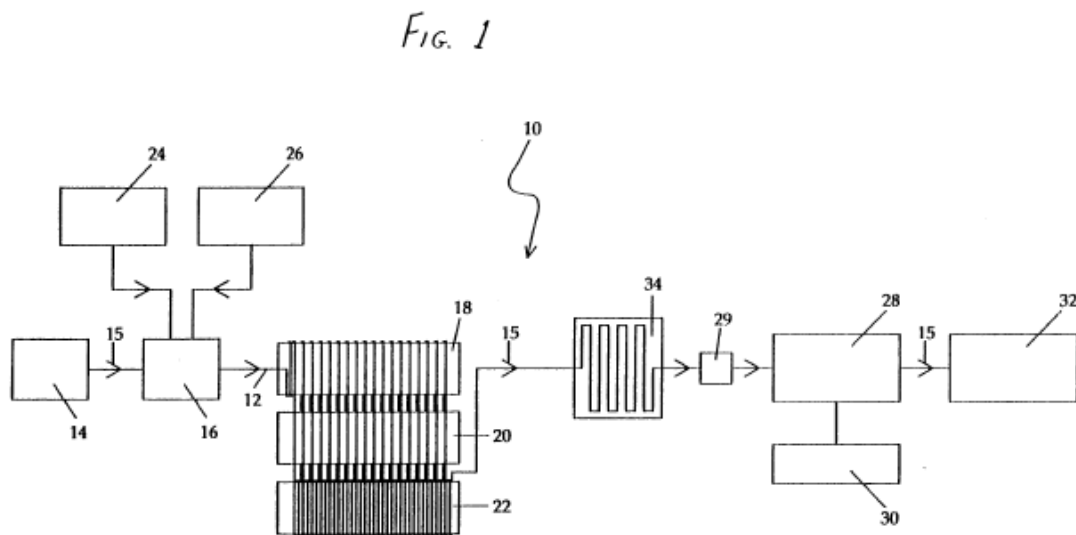
428. Quake even describes performing a specific type of reaction involving a biological molecule inside a droplet:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (e.g. fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

429. While it is my opinion that Quake discloses a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one

biological molecule, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

430. It also would have been obvious to form a plug comprising at least one biological

molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

431. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through

entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

432. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

433. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

434. Quake satisfies this limitation. For example, Quake discloses the conditions required for the enzymatic reaction described above:

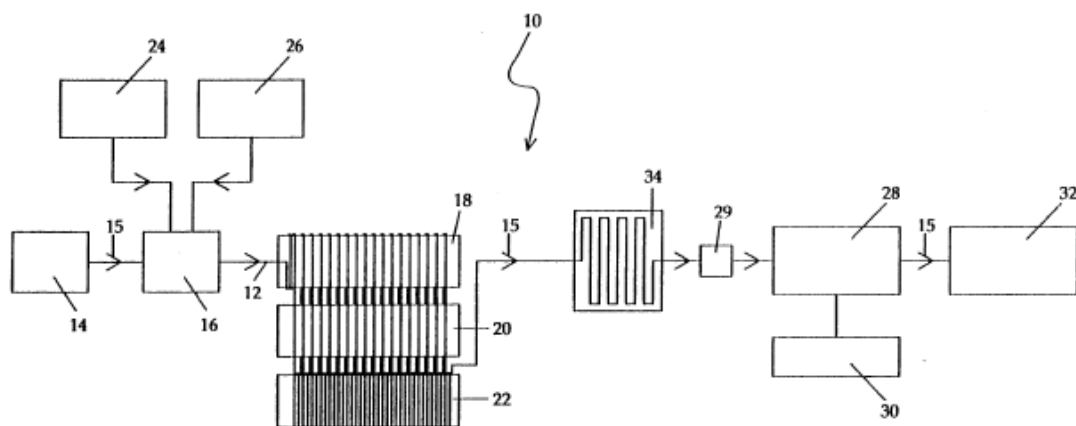
In another embodiment, cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to

catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170].

435. While it is my opinion that Quake discloses providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

436. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold,

and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

437. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

438. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

439. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

440. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

441. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes

(i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules (for example, polynucleotides*, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added). Quake defines “polynucleotide” as including “*double and single stranded RNA and DNA*.” Quake at [0052] (emphasis added).

442. Quake also discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). A person of ordinary skill in the art would have known that PCR requires sample DNA as a reagent. Mullis at Abstract, 2:63-3:1.

(iii) *Claim 3*

443. Claim 3 of the ’407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

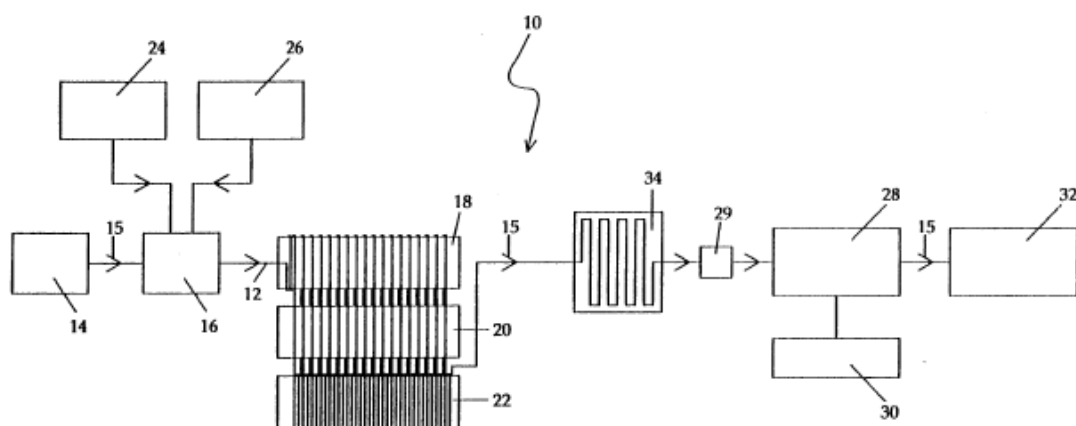
444. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

445. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the ’407 patent explains, PCR is a type of autocatalytic reaction. *See* ’407 patent at 45:30-33 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

446. While it is my opinion that Quake discloses an autocatalytic reactoin, it also would have been obvious to combine the teachings of Quake with one or more prior art

references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

447. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

448. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

449. It also would have been obvious to conduct an autocatalytic reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

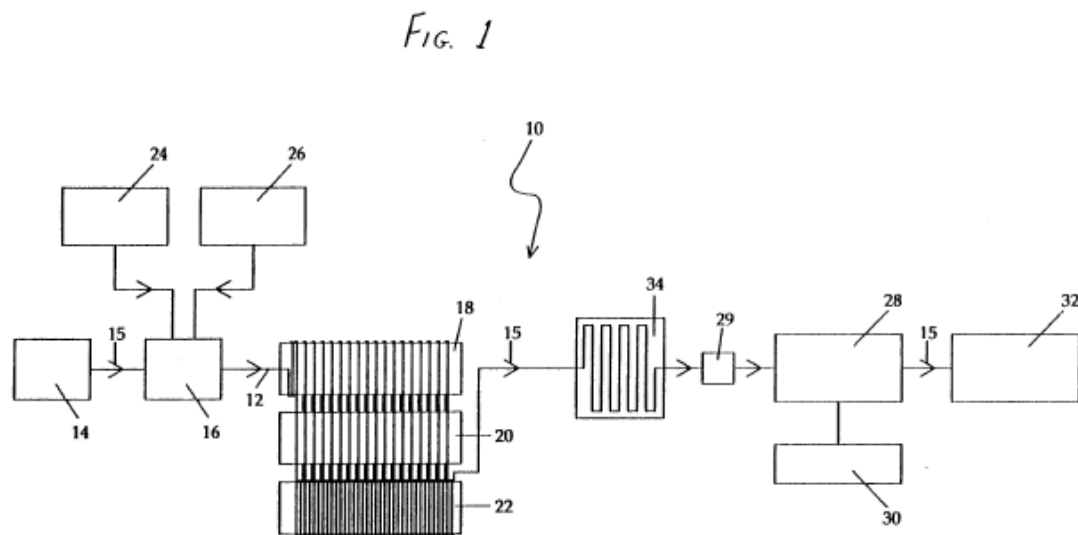
450. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

451. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

452. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

453. While it is my opinion that Quake discloses a polymerase-chain reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain

reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

454. It also would have been obvious to conduct a polymerase-chain reaction in view

of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

455. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns

(1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

456. It also would have been obvious to conduct a polymerase-chain reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

457. Claim 5 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

458. Claim 5 further recites: “**the reaction is an enzymatic reaction.**”

459. Quake satisfies this limitation. For example, Quake discloses that “[i]n various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, *enzymes*, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

460. Quake also describes that the reaction that takes place within a droplet may be an enzymatic reaction. In describing the detection of the results of a reaction, Quake discloses that “[a]s each droplet passes into the detection region, it is examined for a predetermined characteristic (i.e., using the detector) and a corresponding signal is produced . . . the amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the strength of the signal may indicate the size of a molecule, or *the potency or*

amount of an enzyme expressed by a cell, or a positive or negative reaction such as binding or hybridization of one molecule to another, *or a chemical reaction of a substrate catalyzed by an enzyme.*” Quake at [0078] (emphasis added); *see also* Quake at [0106] (emphasis added) (“Enzymes can be analyzed and/or sorted by the extent to which they catalyze chemical reaction of a substrate (conversely, substrate can be analyzed and/or sorted by the level of chemical reactivity catalyzed by an enzyme).”).

461. Quake described a particular enzymatic reaction to detect for the presence of a particular enzyme in sample cells. For example, Quake disclosed:

In another embodiment, cells may produce a reporter *in vivo* (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, *cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene)* with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (e.g. fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

462. Quake even provided “an exemplary channel design for compartmentalization of Enzyme and Substrate.” Quake at [0045] (referring to Fig. 22). Figure 22 is reproduced below:

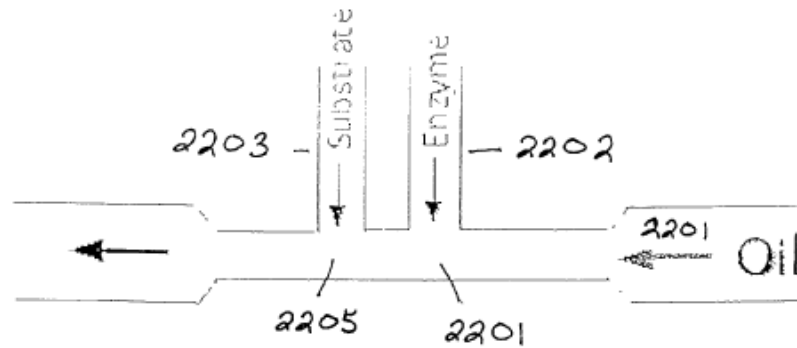
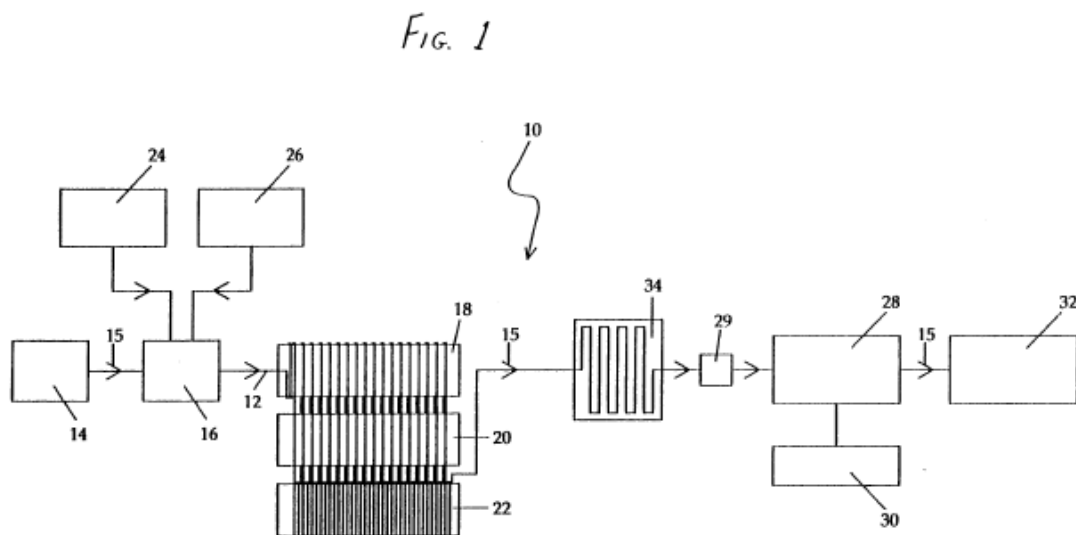


FIG. 22

463. While it is my opinion that Quake discloses a enzymatic reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understood that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via

inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

464. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a

passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

465. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

466. It also would have been obvious to conduct an enzymatic reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

467. Claim 8 of the ’407 patent is dependent on claim 1. I incorporate by reference my

analysis with respect to claim 1.

468. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

469. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within *microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0014] (“In a preferred embodiment, water droplets are extruded into a flow of oil”); Quake at [0015] (“For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).”); Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane or hexadecane)”; Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane)”).

470. Quake also explains that the carrier fluid, or “extrusion fluid,” is “incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel.” Quake at [0022].

471. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

(vii) *Claim 9*

472. Claim 9 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

473. Claim 9 further recites: “**the oil comprises a surfactant.**”

474. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

475. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel . . . The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

476. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating

which minimizes adhesion . . . Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

477. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(viii) *Claim 10*

478. Claim 10 of the ’407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

479. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

480. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

481. While it is my opinion that Quake discloses a fluorosurfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorosurfactant with

microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

482. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

483. It also would have been obvious to use a fluorosurfactant based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

484. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

485. Claim 11 further recites: “**the oil is a fluorinated oil.**”

486. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or*

fluorononane.”).

487. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

488. While it is my opinion that Quake discloses a fluorinated oil, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

489. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

490. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for

carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

491. It also would have been obvious to use a fluorinated oil based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

492. Claim 13 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

493. Claim 13 further recites: “**the providing step includes heating.**”

494. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Quake at [0080]. A person of skill in the art would have known that the PCR reaction required cycles of heating and cooling. Mullis at 9:55-60.

495. While it is my opinion that Quake discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the

invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

496. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

497. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

498. It also would have been obvious to provide heating to the microfluidic system based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(b) Invalidity Based on Shaw Stewart

499. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art

references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

500. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

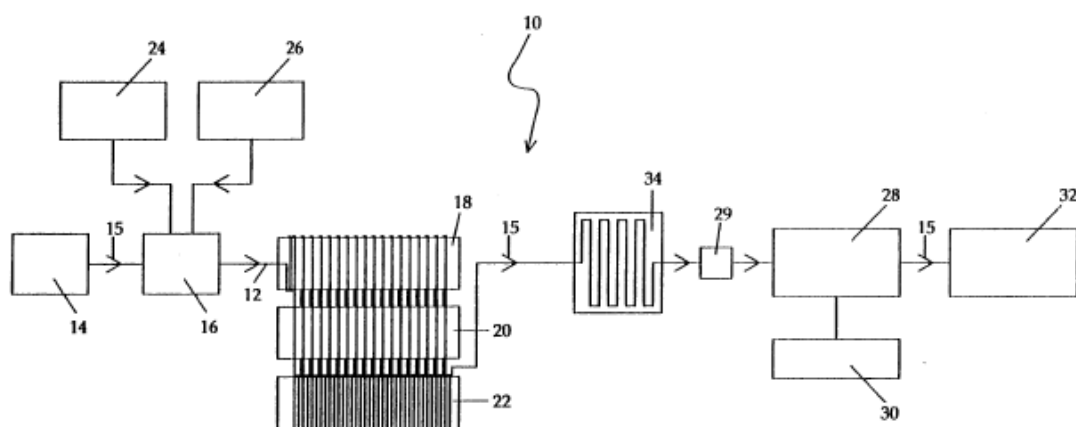
501. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

502. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

503. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett, at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also

describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

504. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally, at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the

amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

505. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

506. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang, at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

507. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

508. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

509. Shaw Stewart satisfies this claim limitation. For example, Shaw Stewart described that a microfluidic system with at least two channels comprising at least one junction, as shown in Figure 1, reproduced below:

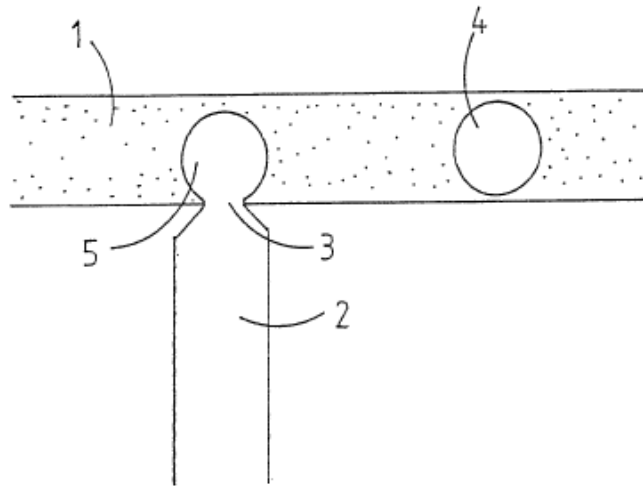


Figure 1.

Shaw Stewart at Fig. 1. In describing the figure, Shaw Stewart stated that “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

510. Figure 2 of Shaw Stewart, reproduced below, also discloses this limitation.

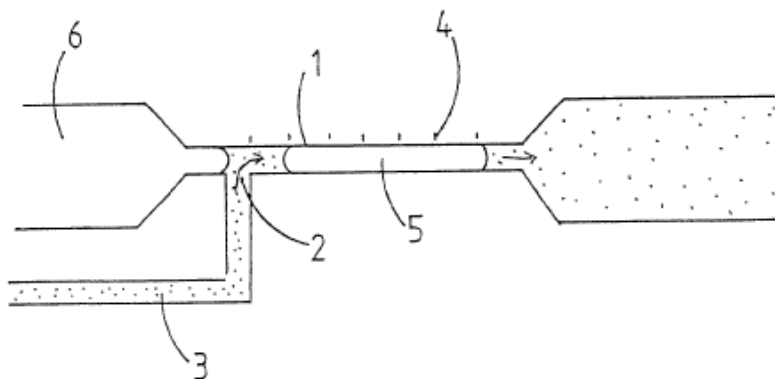


Figure 2.

Shaw Stewart at Fig. 2. In describing Figure 2, Shaw Stewart stated that “[i]n this case the reagent (6) is passed into a tube of considerably narrower bore (1) than the cross-section of the

droplets to be produced. The tube is graduated relative to the opening (2) of a side arm (3). The reagent is passed into the tube to a certain graduation (4), whereupon carrier phase is introduced from the side-arm, thus breaking off a droplet to the required size.” Shaw Stewart at 1:96-104.

511. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

512. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

513. Figure 1 of Shaw Stewart also discloses this limitation.

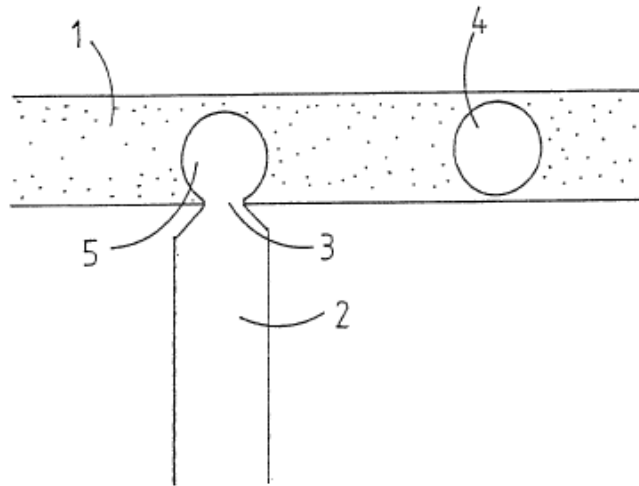


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

514. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an

immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

515. While it is my opinion that Shaw Stewart discloses flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

516. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological

molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally, at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

517. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

518. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological

molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang, at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

519. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

520. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

521. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible

liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

522. Figure 1 of Shaw Stewart also discloses this limitation.

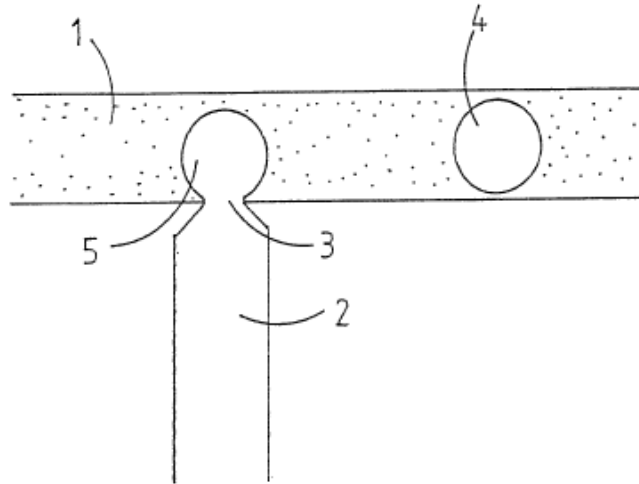


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

523. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I

while ignoring other portions that directly contradicted PO's arguments. For example, one of PO's primary arguments was that Stewart I only disclosed introducing fluid in a 'stepwise fashion,' whereas in the '503 Patent droplets are formed by 'combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.' In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

524. Claim 1 further recites: **"forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels."**

525. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

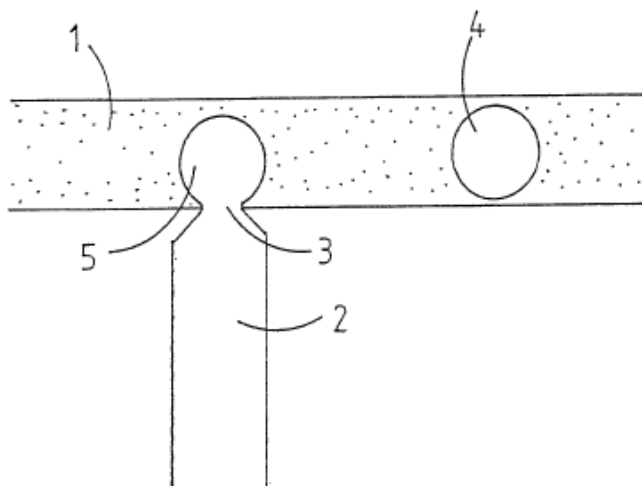


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

526. Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

527. While it is my opinion that Shaw Stewart discloses forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, it also would have been obvious to combine the teachings of Shaw Stewart

with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett, at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

528. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally, at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

529. It also would have been obvious to form at least one plug of the aqueous fluid

containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

530. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang, at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

531. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the

aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

532. Claim 1 further recites: “**the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.**”

533. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

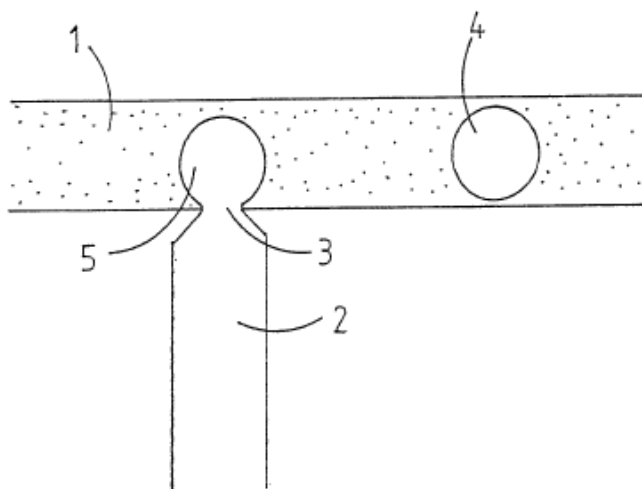


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

534. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with

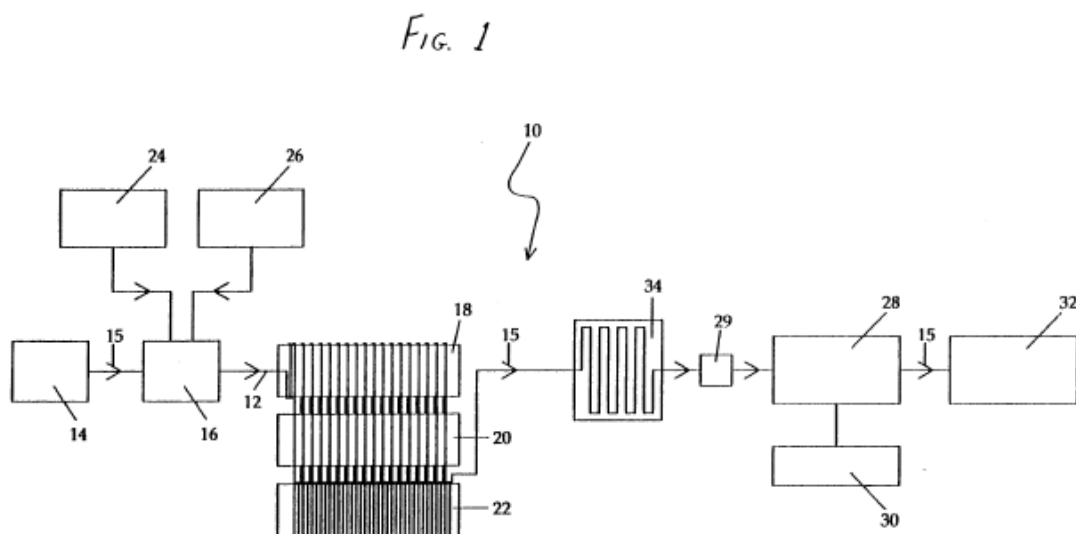
diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

535. Claim 1 further recites: **“wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.”**

536. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Because Shaw Stewart disclosed that his invention could be applied to, for example, biochemistry and recombinant DNA work, the droplets formed in Shaw Stewart would have been comprised at least one biological molecule and at least one reagent for conducting the reaction with the at least biological molecule.

537. While it is my opinion that Shaw Stewart discloses a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett, at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume,

approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

538. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally, at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

539. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

540. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references

cited therein.

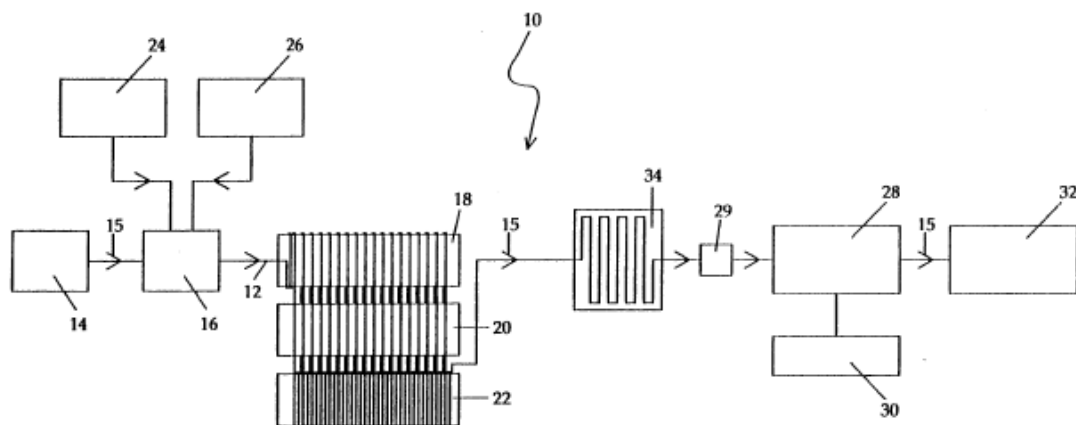
541. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

542. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would allow different types of reactions to take place within droplets. Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

543. While it is my opinion that Shaw Stewart discloses providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett, at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention

relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

544. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of

single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally, at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

545. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA

would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

546. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

547. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

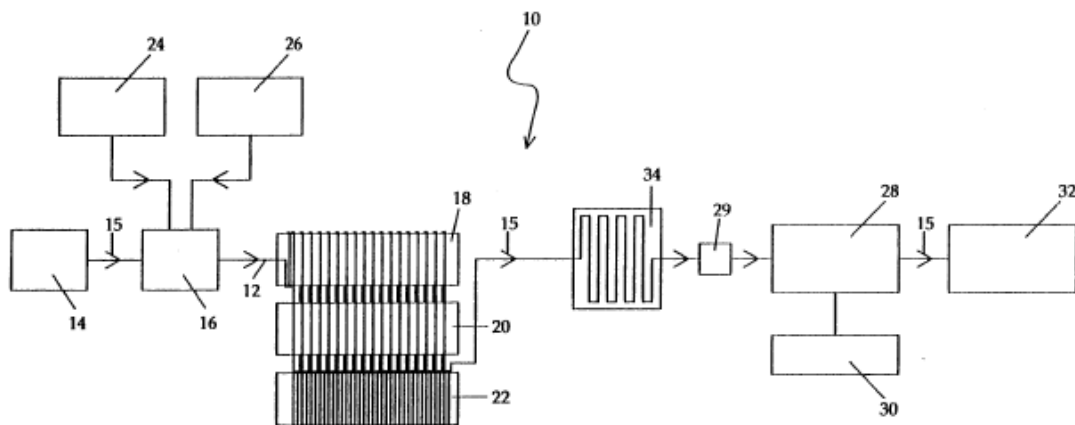
548. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

549. Shaw Stewart satisfies this limitation. For example, Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added). Because Shaw Stewart disclosed that his invention could be applied to, for example, recombinant DNA work, the droplets formed in Shaw Stewart would have been comprised at least one DNA molecule.

550. While it is my opinion that Shaw Stewart discloses that the at least one biological molecule is DNA or RNA, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of

carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

551. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single

DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

552. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

553. I also incorporate by reference my opinions discussing the disclosure of this claim limitation in Quake. *See supra* ¶¶ 439-442. It would have been obvious to combine the teachings of Quake with the teachings of Shaw Stewart.

554. It also would have been obvious that the at least one biological molecule is DNA or RNA based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

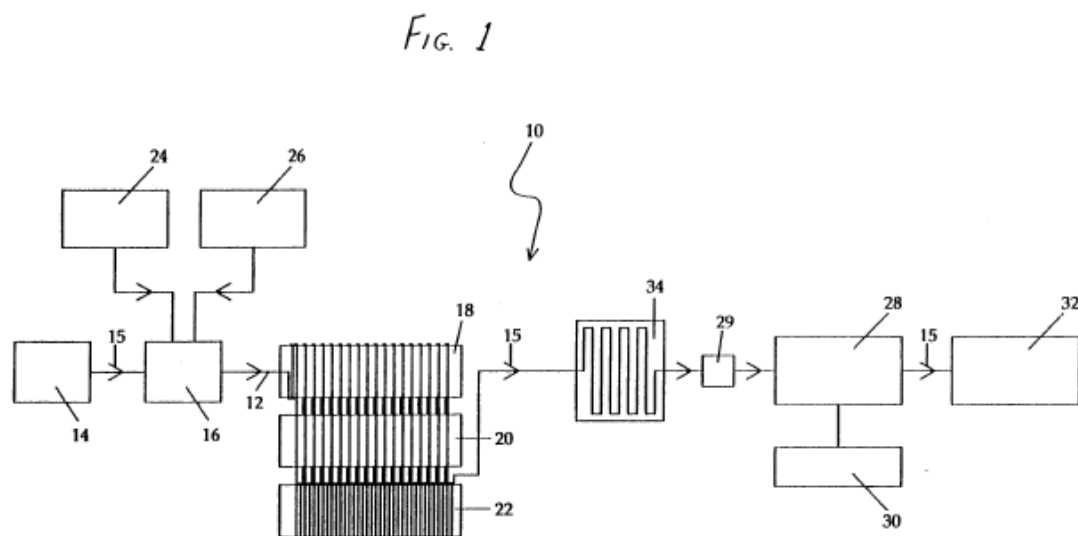
(iii) *Claim 3*

555. Claim 3 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

556. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

557. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s)

to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

558. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for

operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

559. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

560. It also would have been obvious to conduct an autocatalytic reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections

VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

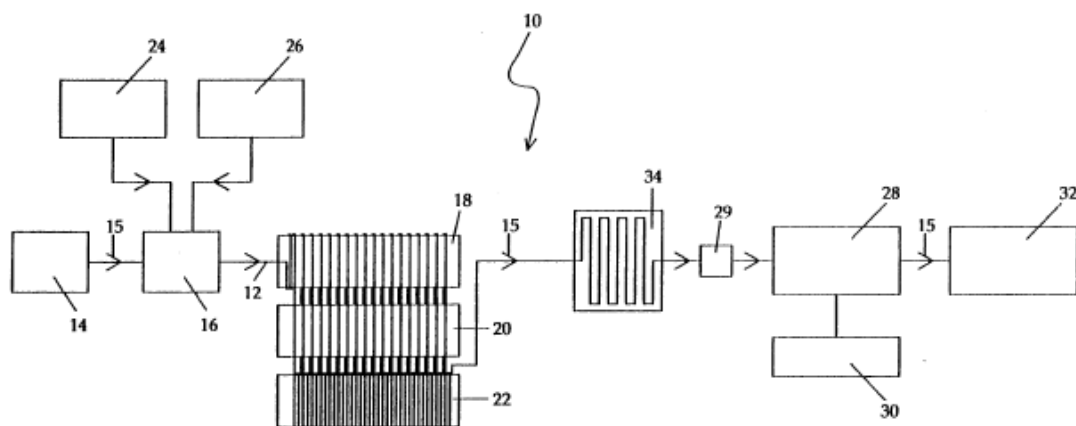
(iv) *Claim 4*

561. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

562. Claim 4 further recites: **“the reaction is a polymerase chain reaction.”**

563. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

564. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

565. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

566. It also would have been obvious to conduct a polymerase-chain reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

567. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

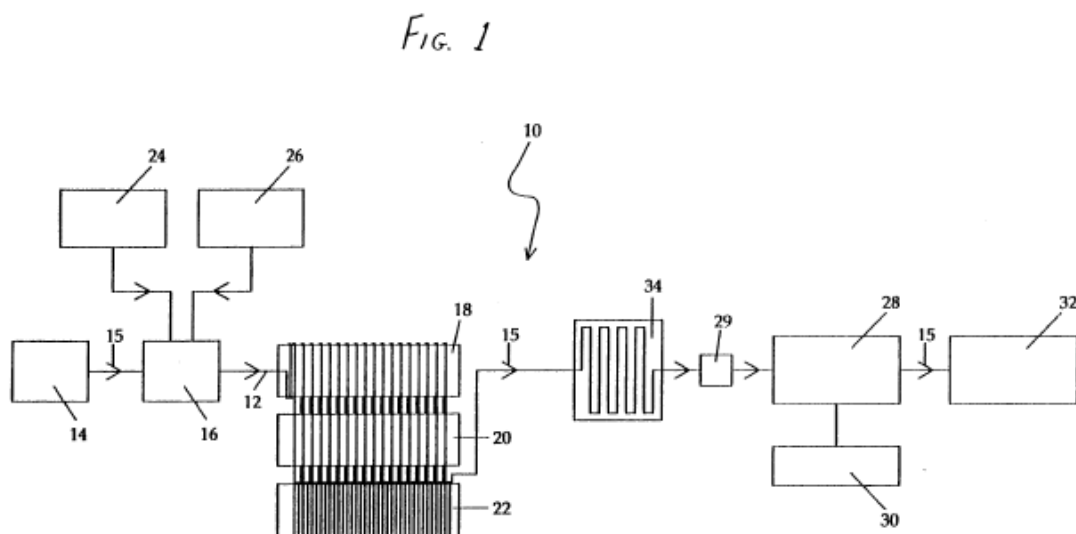
568. Claim 5 further recites: “**the reaction is an enzymatic reaction.**”

569. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. In order for the inventions described in Shaw Stewart to be applied to “recombinant DNA work,” enzymatic reactions must take place with Shaw Stewart’s droplets. *See generally*, Loenen.

570. While it is my opinion that Shaw Stewart discloses a enzymatic reaction, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understand that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is

injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

571. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from

the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

572. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

573. It also would have been obvious to conduct an enzymatic reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

574. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

575. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

576. Shaw Stewart satisfies this limitation. For example, Shaw Stewart states that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

(vii) *Claim 9*

577. Claim 9 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

578. Claim 9 further recites: “**the oil comprises a surfactant.**”

579. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxyol, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains **a surfacting agent** which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(viii) *Claim 10*

580. Claim 10 of the '407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

581. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

582. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorosurfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

583. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

584. It also would have been obvious to use a fluorosurfactant based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

585. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

586. Claim 11 further recites: “**the oil is a fluorinated oil.**”

587. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses

that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.” Shaw Stewart II at 4 (emphasis added).

588. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

589. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

590. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects.

Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

591. It also would have been obvious to use a fluorinated oil based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

592. Claim 13 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

593. Claim 13 further recites: “**the providing step includes heating.**”

594. Shaw Stewart satisfies this limitation. For example, Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

595. While it is my opinion that Shaw Stewart discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also

describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

596. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

597. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

598. It also would have been obvious to provide heating to the microfluidic system based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(c) Invalidity Based on Burns (2001)

599. It is my opinion that Burns (2001) discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art

references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

600. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

601. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

602. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

603. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

604. This reaction is illustrated in Figure 4:

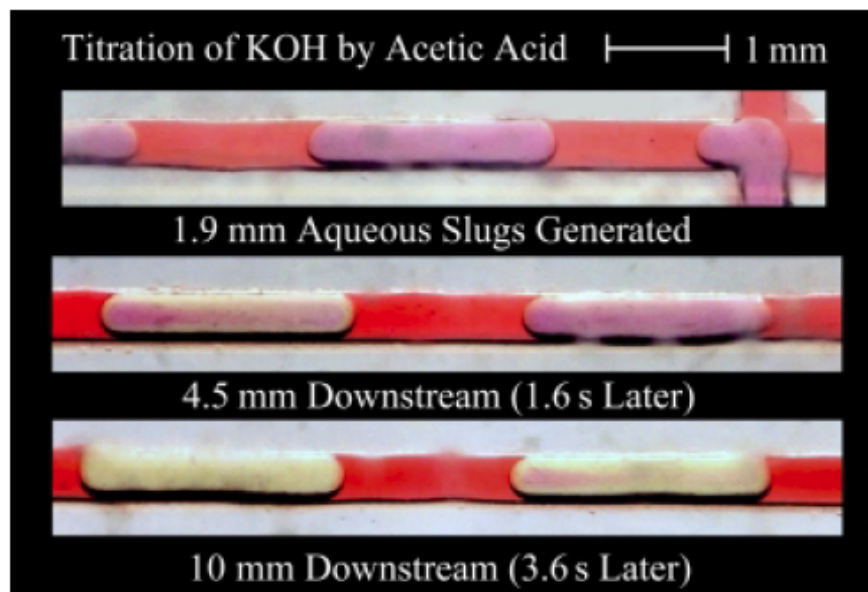
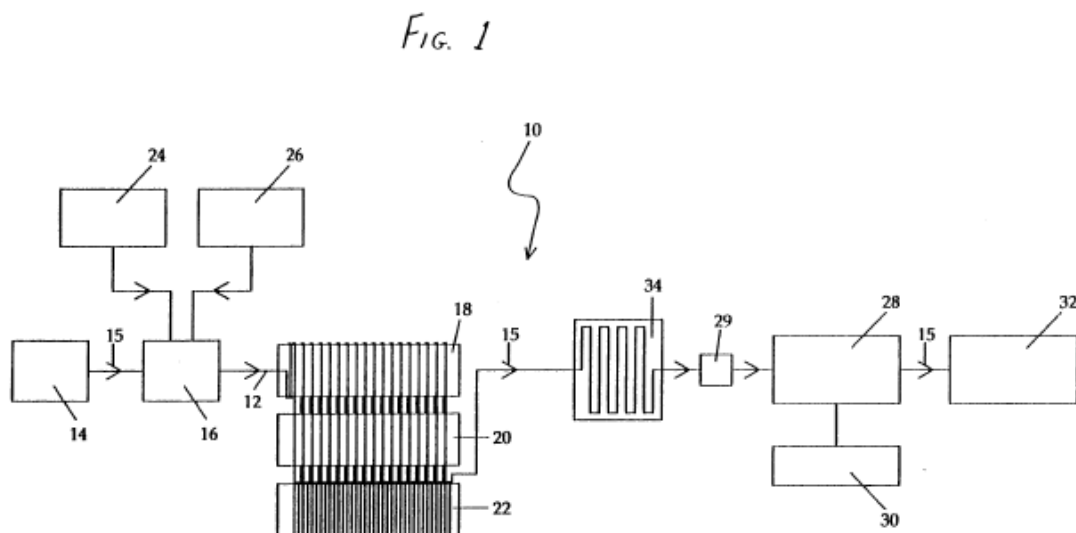


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

605. While it is my opinion that Burns (2001) discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an

enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

606. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system

using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

607. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

608. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer

or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

609. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

610. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

611. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel has been developed. The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a large enhancement in the interfacial mass transfer and the reaction rate. Mass transfer performance data has been obtained for a *glass chip-based reactor in a 380 μm wide channel* by monitoring the extraction of acetic acid from kerosene slugs as they moved along the reactor channel.” Burns (2001) at Abstract (emphasis added).

612. Figure 4 from Burns (2001) also illustrates a microfluidic system with at least two channels having at least one junction:

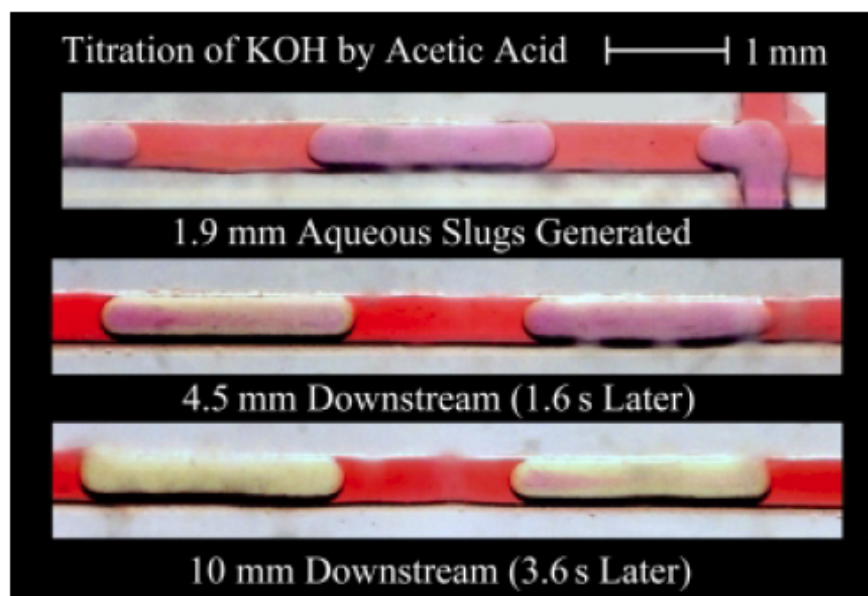


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

613. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

614. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

615. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

616. This reaction is illustrated in Figure 4:

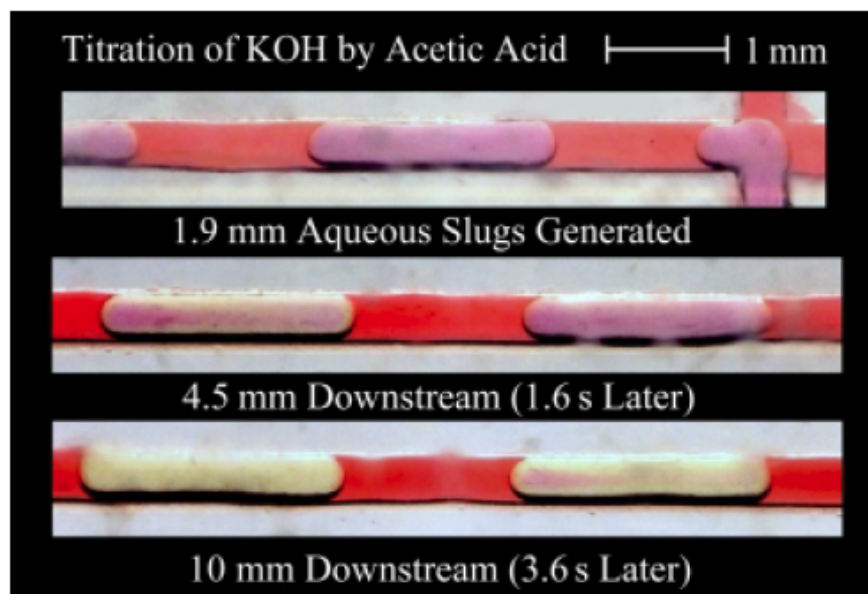


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

617. It would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

618. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the

separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

619. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

620. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

621. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on

Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

622. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

623. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

624. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

625. This reaction is illustrated in Figure 4:

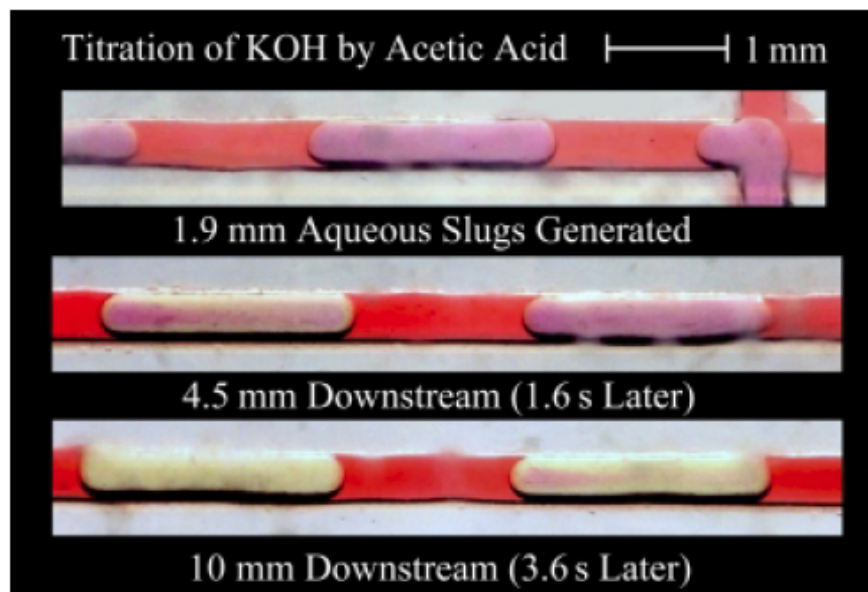


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

626. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.**”

627. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11

(emphasis added).

628. Figure 4 also illustrates this process:

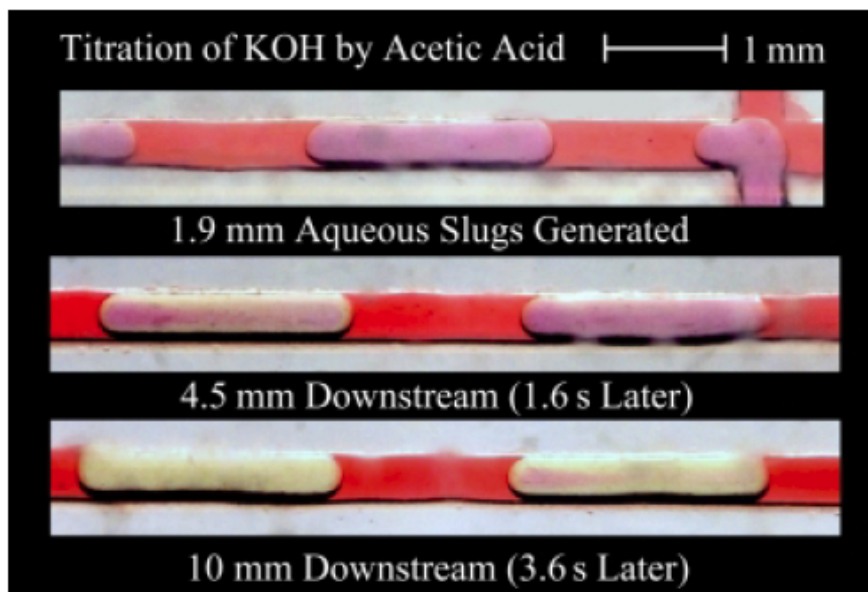


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

629. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

630. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a

microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

631. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

632. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating

solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

633. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

634. Claim 1 further recites: **“the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.”**

635. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

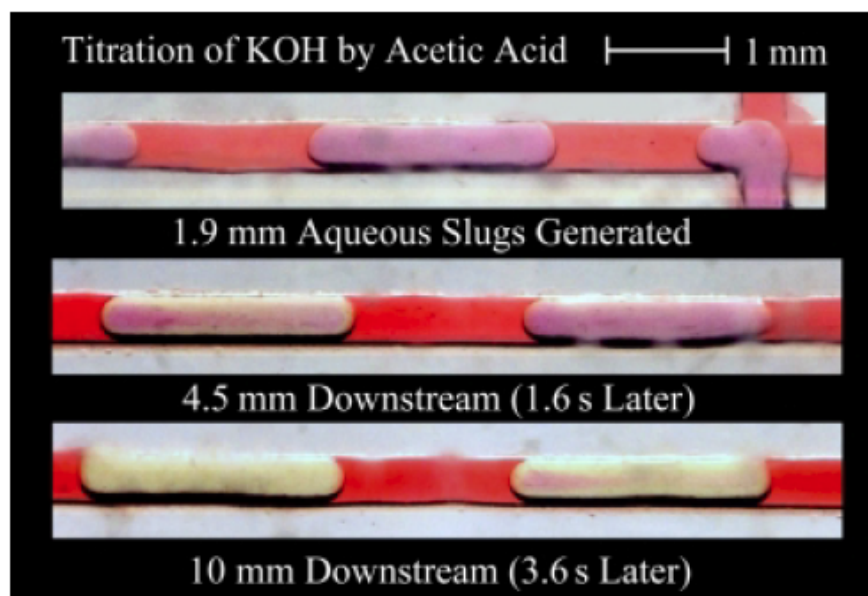


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

636. When a less viscous fluid moves as a “plug” through a more viscous fluid in a micro-channel and when the more viscous film forms a film around the “plug,” the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

637. Claim 1 further recites: “**wherein the at least one plug comprises at least one**

biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.”

638. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

639. This reaction is illustrated in Figure 4:

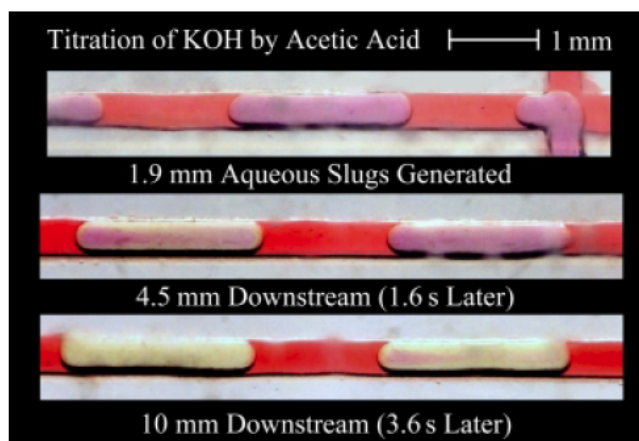
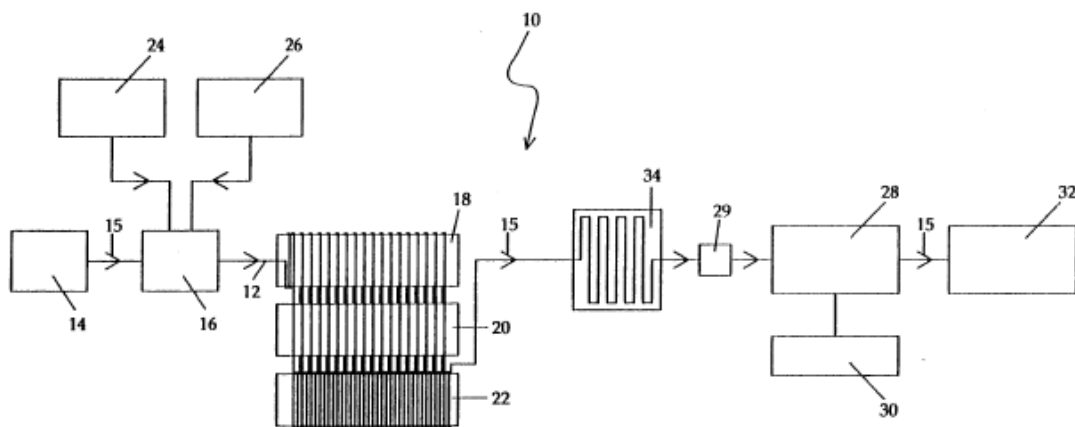


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

640. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

641. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

642. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only

local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

643. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

644. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

645. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) provides conditions suitable for an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which

was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. ***Kerosene was used as the basis of the organic phase*** with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

646. This reaction is illustrated in Figure 4:

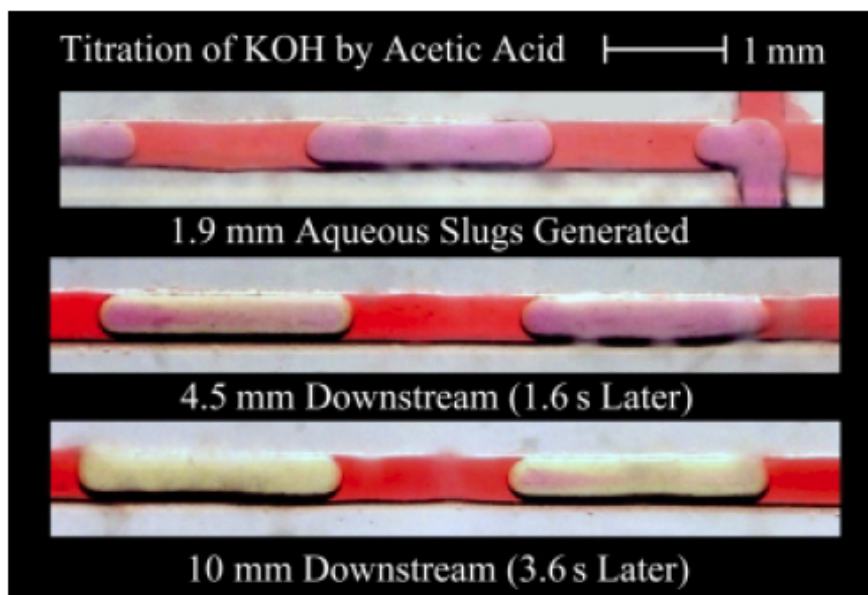
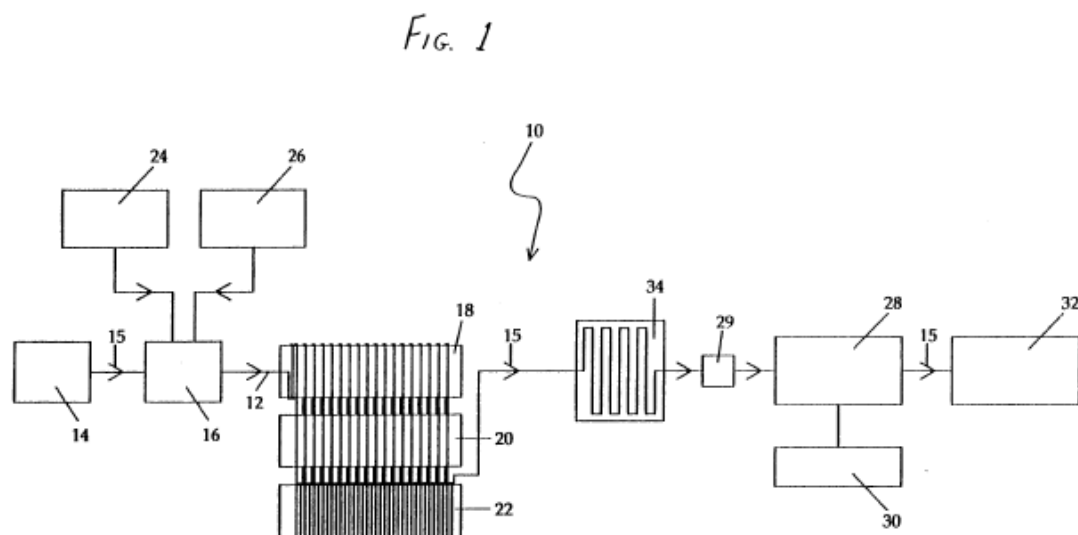


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

647. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

648. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place

on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

649. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR

amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

650. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

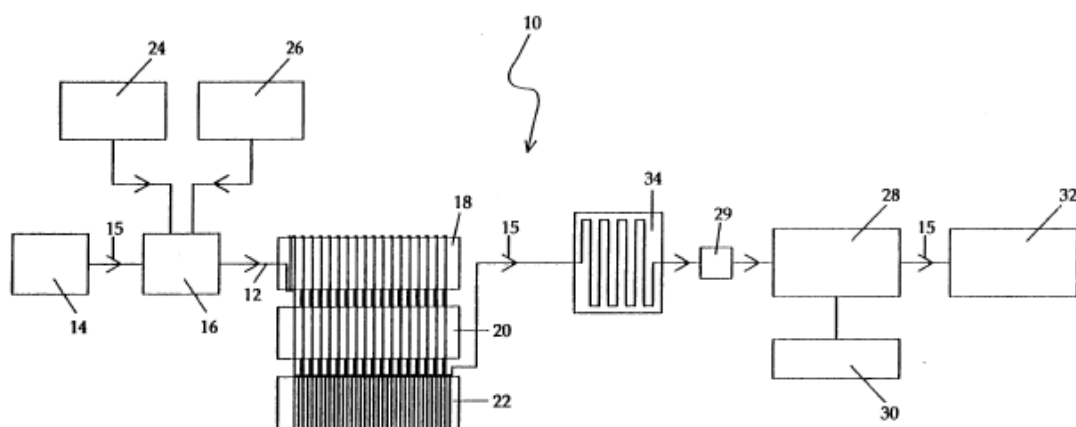
651. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

652. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

653. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

654. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system

using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

655. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

656. I also incorporate by reference my opinions discussing the disclosure of this claim limitation in Quake. *See supra* ¶¶ 439-442. It would have been obvious to combine the teachings of Quake with the teachings of Burns (2001).

657. It also would have been obvious that the at least one biological molecule is DNA or RNA based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art),

and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

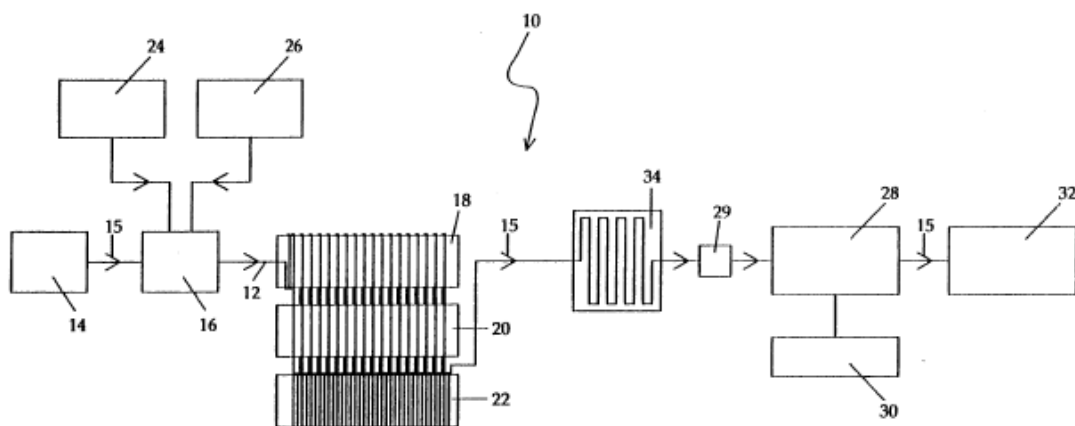
(iii) *Claim 3*

658. Claim 3 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

659. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

660. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

661. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

662. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

663. It also would have been obvious to conduct an autocatalytic reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

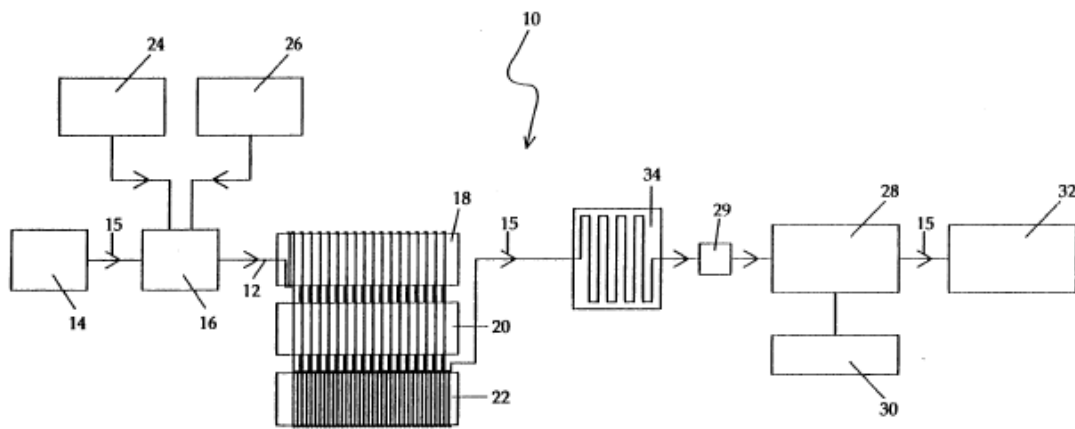
(iv) *Claim 4*

664. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

665. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

666. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

667. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

668. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

669. It also would have been obvious to conduct a polymerase-chain reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

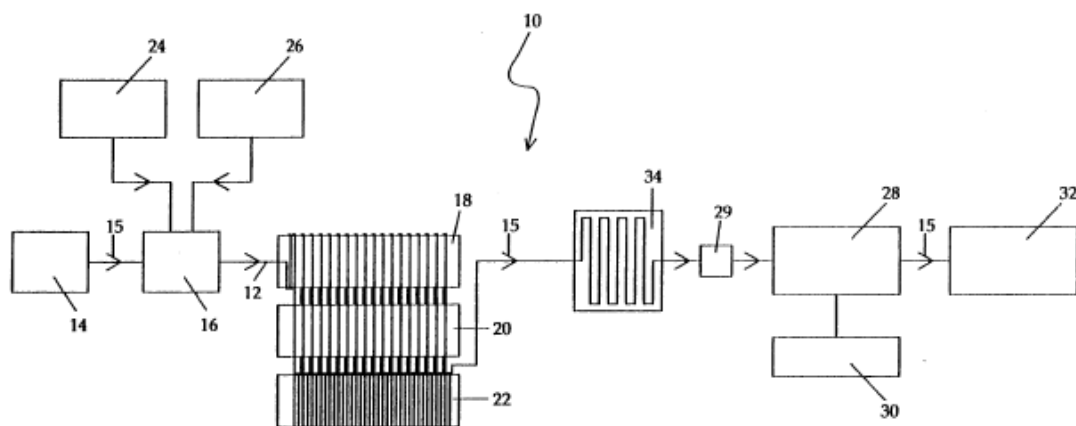
(v) *Claim 5*

670. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

671. Claim 5 further recites: **“the reaction is an enzymatic reaction.”**

672. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understood that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

673. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

674. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

675. It also would have been obvious to conduct an enzymatic reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

676. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

677. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

678. Burns (2001) satisfies this limitation. For example, Burns (2001) makes clear that the carrier fluid is an oil. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

(vii) *Claim 9*

679. Claim 9 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

680. Claim 9 further recites: “**the oil comprises a surfactant.**”

681. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

682. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

683. It also would have been obvious to use a surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 10*

684. Claim 10 of the '407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

685. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

686. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorosurfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

687. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract.

Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

688. It also would have been obvious to use a fluorosurfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

689. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

690. Claim 11 further recites: “**the oil is a fluorinated oil.**”

691. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

692. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that

these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

693. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

694. It also would have been obvious to use a fluorinated oil based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

695. Claim 13 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

696. Claim 13 further recites: “**the providing step includes heating.**”

697. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.”

Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

698. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

699. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

700. It also would have been obvious to provide heating to the microfluidic system based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(d) Invalidity Based on Nisisako

701. It is my opinion that Nisisako discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge

of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

702. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

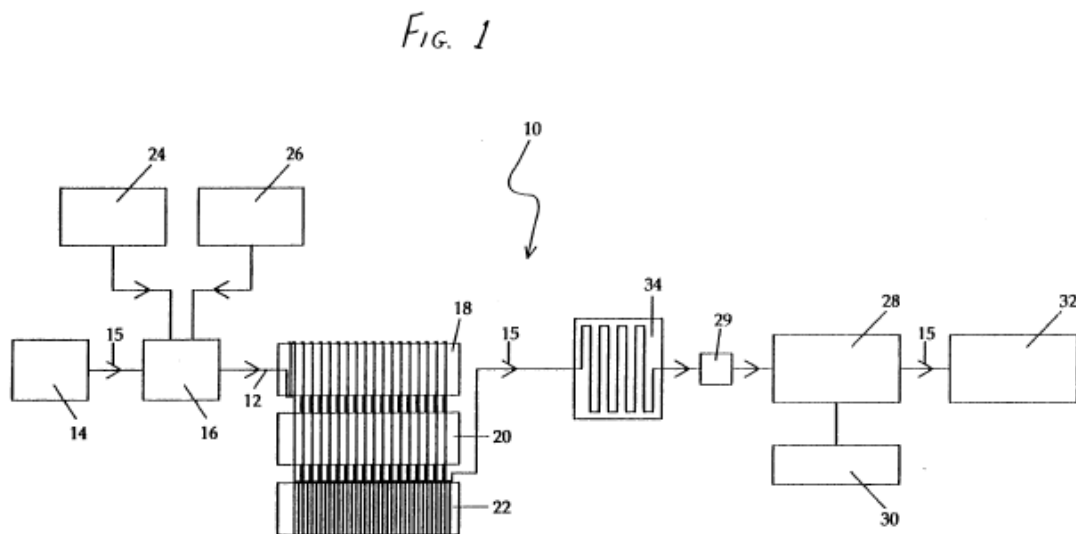
703. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

704. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network*.” Nisisako at Abstract (emphasis added).

705. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

706. While it is my opinion that Nisisako discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of

Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

707. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

708. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-

tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

709. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

710. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

711. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

712. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep.*” Nisisako at Abstract (emphasis added).

713. The figures in Nisisako also disclose this limitation. For example, Figures 1 and 2 both show a microfluidic system with two channels meeting at a junction:

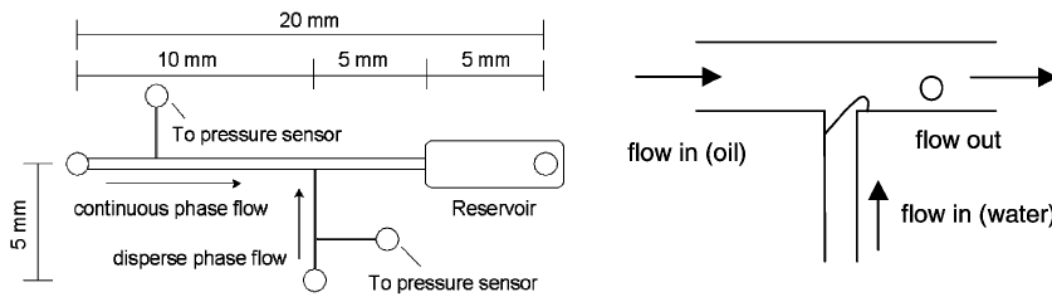


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

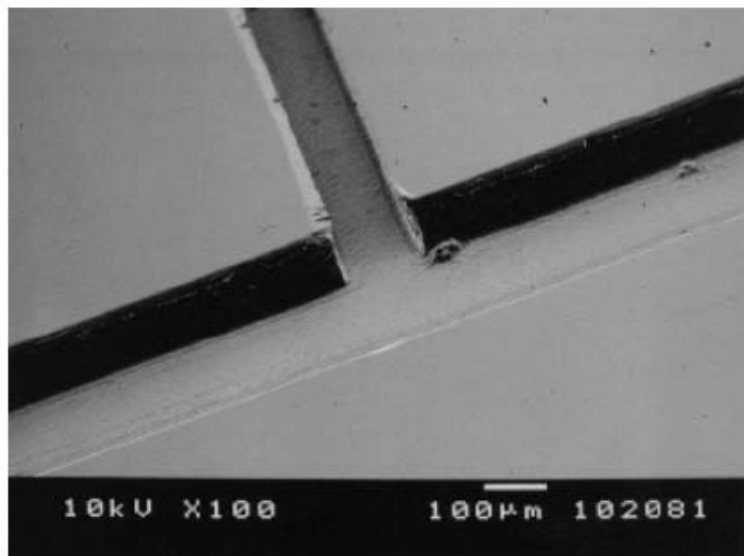


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

714. Claim 1 further recites: “**continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.**”

715. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps.* No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that *the flow rate is constant.*”).

716. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

717. While it is my opinion that Nisisako discloses flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in

a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

718. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

719. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological

molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

720. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

721. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

722. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

723. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that *the flow rate is constant.*”).

724. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.**”

725. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to

study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating water-in-oil droplets in a microchannel network.”).

726. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

727. Nisisako also discloses that “[t]his method of droplet formation is shown schematically in Fig. 1.” Nisisako at 24. Figure 1 is reproduced below:

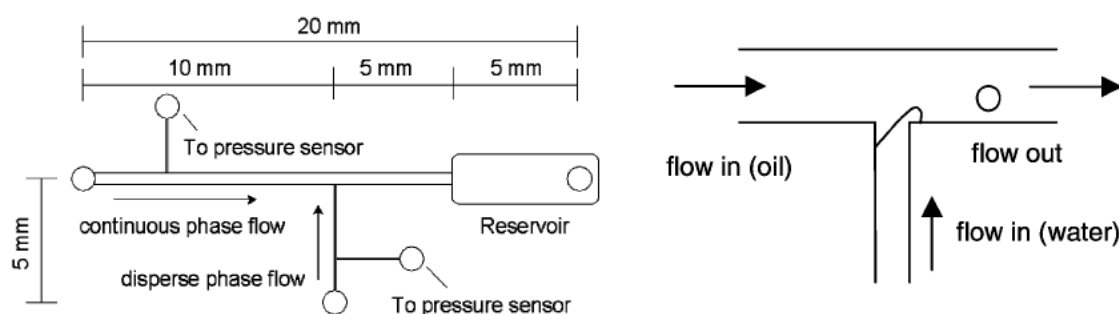


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

Nisisako at Fig. 1. Droplet formation at the T-junction is also illustrated by Figure 3:

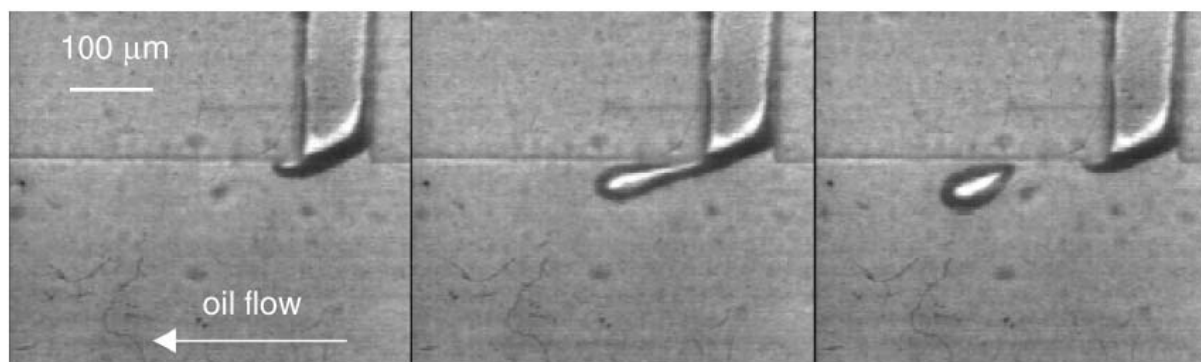


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Fig. 3; *see also* Nisisako at 25 (“Regular-sized droplets of water in oil were generated at the T-junction (Fig. 3).”

728. While it is my opinion that Nisisako discloses forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

729. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

730. It also would have been obvious to form at least one plug of the aqueous fluid

containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

731. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

732. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited

therein.

733. Claim 1 further recites: “**the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.**”

734. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by the oil:

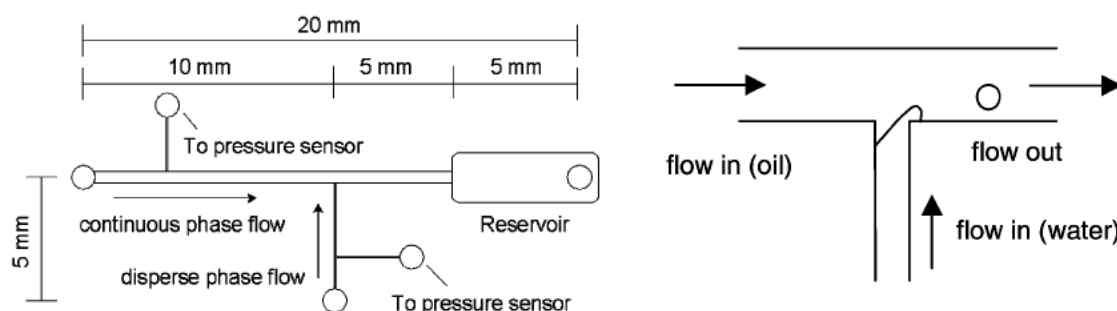


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

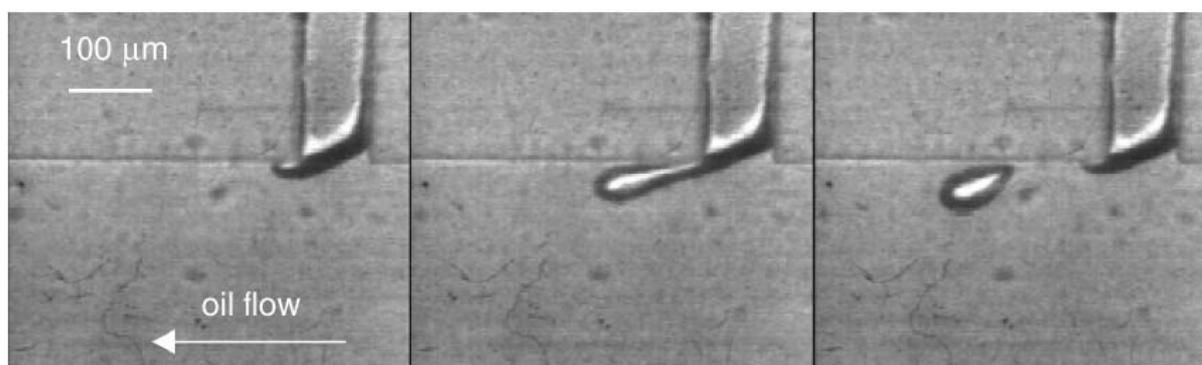


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

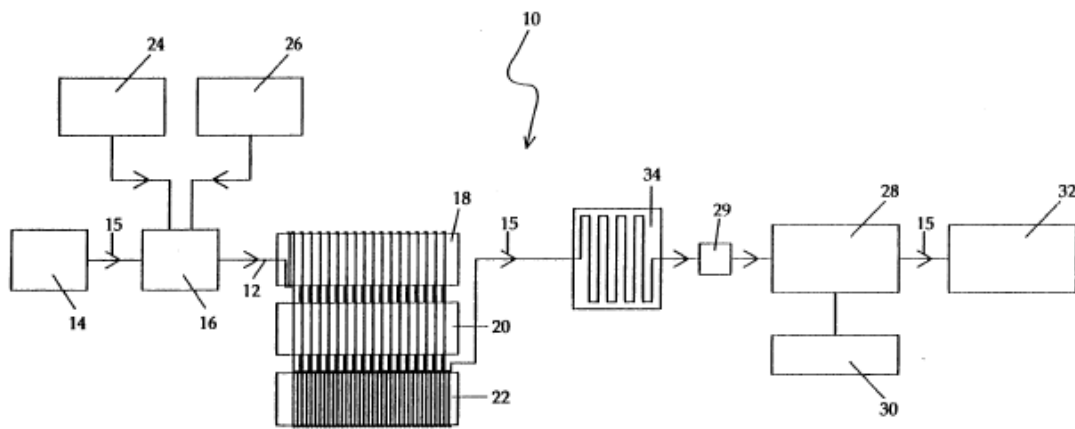
Nisisako at Figs. 1 and 3.

735. Claim 1 further recites: “**wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.**”

736. Nisisako satisfies this limitation. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

737. While it is my opinion that Nisisako discloses a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

738. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

739. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

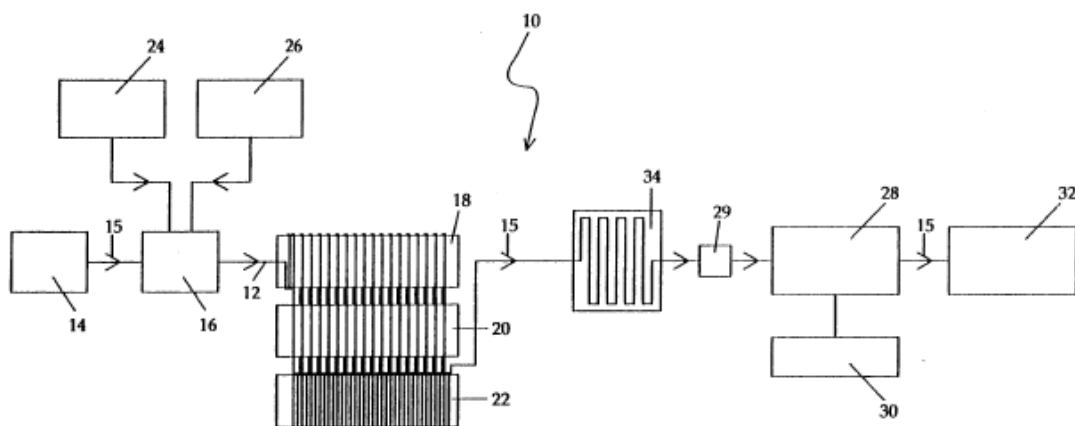
740. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

741. Claim 1 further recites: “**and providing conditions suitable for the reaction in**

the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.”

742. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

743. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

744. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

745. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

746. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my

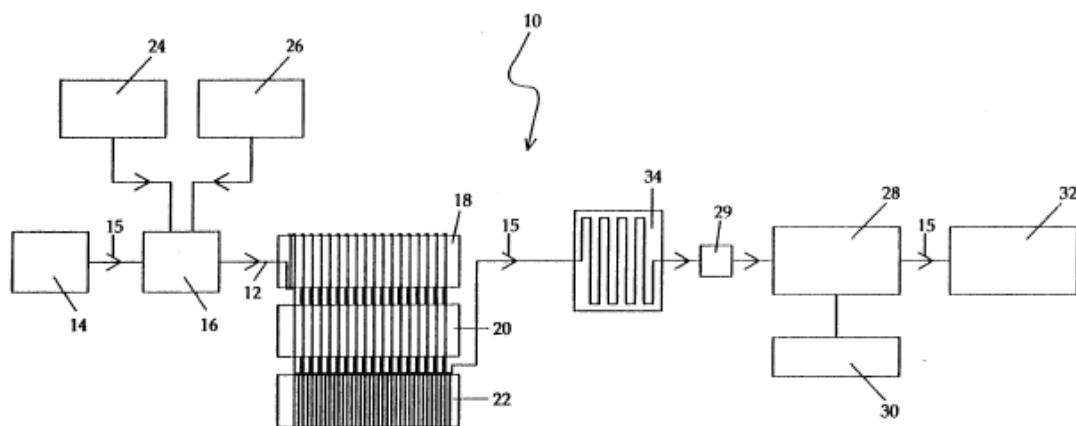
analysis with respect to claim 1.

747. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

748. Nisisako satisfies this limitation. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

749. While it is my opinion that Nisisako discloses that the at least one biological molecule is DNA or RNA, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

750. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was

opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

751. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

752. I also incorporate by reference my opinions discussing the disclosure of this claim limitation in Quake. *See supra* ¶¶ 439-442. It would have been obvious to combine the teachings of Quake with the teachings of Nisisako.

753. It also would have been obvious that the at least one biological molecule is DNA or RNA based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

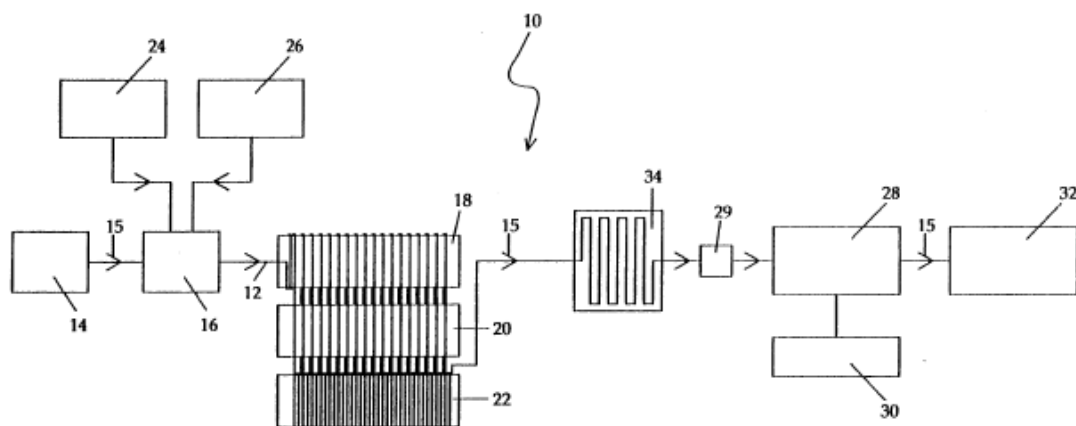
754. Claim 3 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

755. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

756. Nisisako at least renders obvious this limitation, in light of the background

knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

757. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

758. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

759. It also would have been obvious to conduct an autocatalytic reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

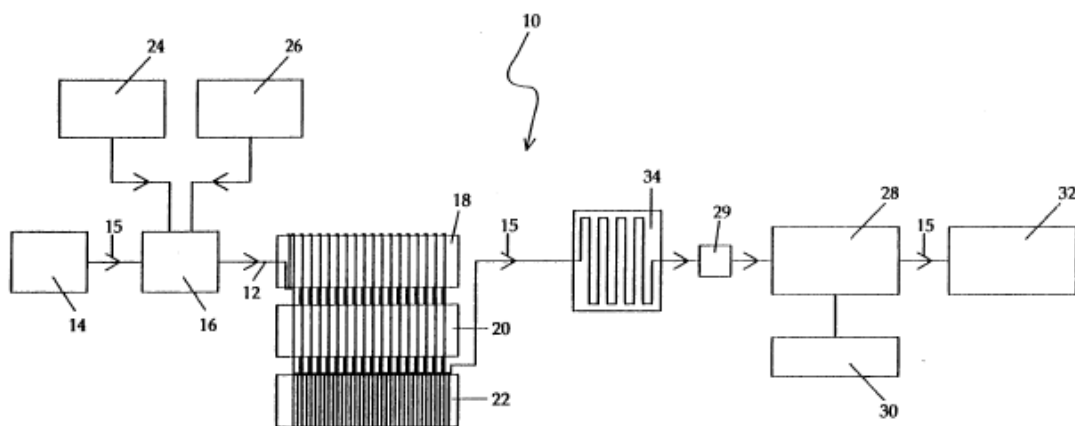
(iv) *Claim 4*

760. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

761. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

762. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

763. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

764. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

765. It also would have been obvious to conduct a polymerase-chain reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

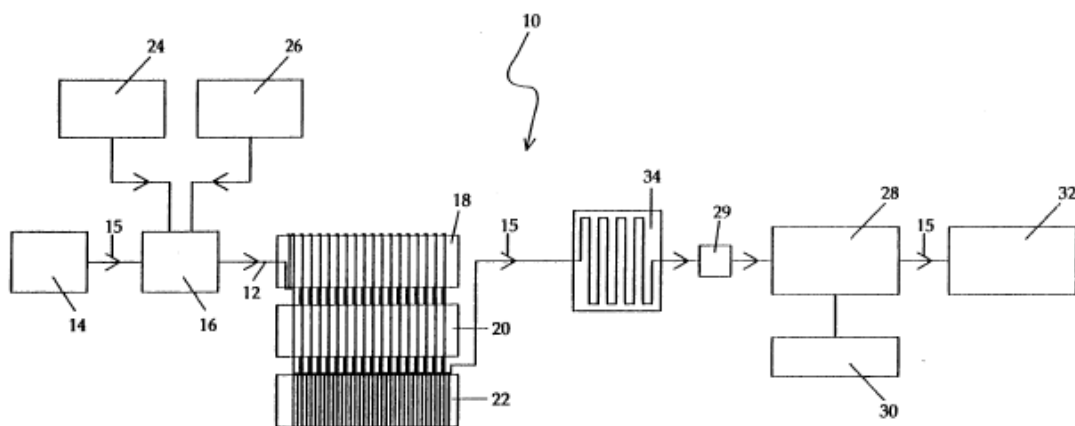
(v) *Claim 5*

766. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

767. Claim 5 further recites: **“the reaction is an enzymatic reaction.”**

768. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understand that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

769. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

770. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

771. It also would have been obvious to conduct an enzymatic reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

772. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

773. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

774. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); see also Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating *water-in-oil* droplets in a microchannel network.”).

(vii) Claim 9

775. Claim 9 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

776. Claim 9 further recites: “**the oil comprises a surfactant.**”

777. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

778. It also would have been obvious to use a surfactant in view of Krafft. Krafft

describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

779. It also would have been obvious to use a surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 10*

780. Claim 10 of the '407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

781. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

782. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorosurfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

783. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that

these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

784. It also would have been obvious to use a fluorosurfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

785. Claim 11 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

786. Claim 11 further recites: “**the oil is a fluorinated oil.**”

787. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

788. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

789. It also would have been obvious to use a fluorinated oil in view of Ramsey.

Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

790. It also would have been obvious to use a fluorinated oil based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

791. Claim 13 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

792. Claim 13 further recites: “**the providing step includes heating.**”

793. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier

fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

794. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

795. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

796. It also would have been obvious to provide heating to the microfluidic system based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(e) Invalidity Based on Thorsen

797. It is my opinion that Thorsen discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

798. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

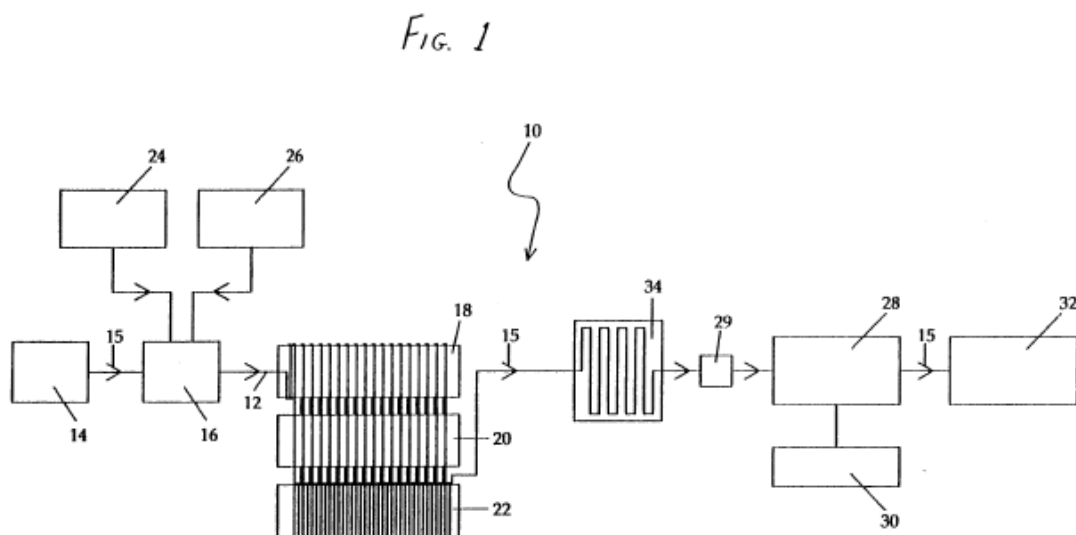
799. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

800. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

801. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik; Chiu, D., et al., “Chemical Transformations in Individual Ultrasmall Biomimetic Containers,” *Science*, 283:1892-1895 (1999) (“Chiu”) (10X-000254987-91); Kung, C.-Y., et al., “Confinement and Manipulation of Individual Molecules in Attoliter Volumes,” *Anal. Chem.* 70: 658-661 (1998) (“Kung”) (10X-000255372-75).

802. While it is my opinion that Thorsen discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular

sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

803. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single

DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

804. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally

controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

805. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

806. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

807. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

808. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at *the junction of two microfluidic channels* containing water and an oil mixture, respectively.” Thorsen at 4163 (emphasis added); *see also* Thorsen at Abstract

(“Here, we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

809. Thorsen also describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring acrylated urethane (Ebecryl 270, UCB Chemicals) on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately $60\text{ }\mu\text{m}$ wide x $9\text{ }\mu\text{m}$ high, tapering to $35\text{ }\mu\text{m}$ x $6.5\text{ }\mu\text{m}$ in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. Figure 1, showing a microfluidic system with two channels and a junction, is shown below:

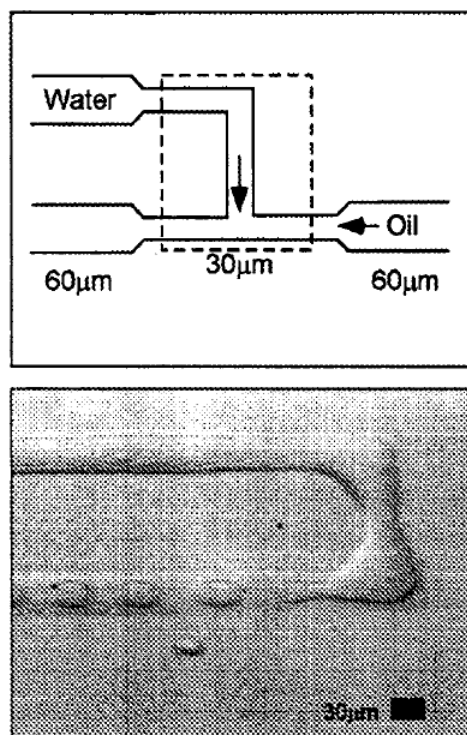


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

810. Claim 1 further recites: **“continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.”**

811. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

812. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

813. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

814. While it is my opinion that Thorsen discloses flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two

channels, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

815. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the

separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

816. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

817. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

818. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on

Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

819. Claim 1 further recites: “**continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.**”

820. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

821. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

822. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.**”

823. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the

presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

824. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

825. Figure 1, showing plug formation, is reproduced below:

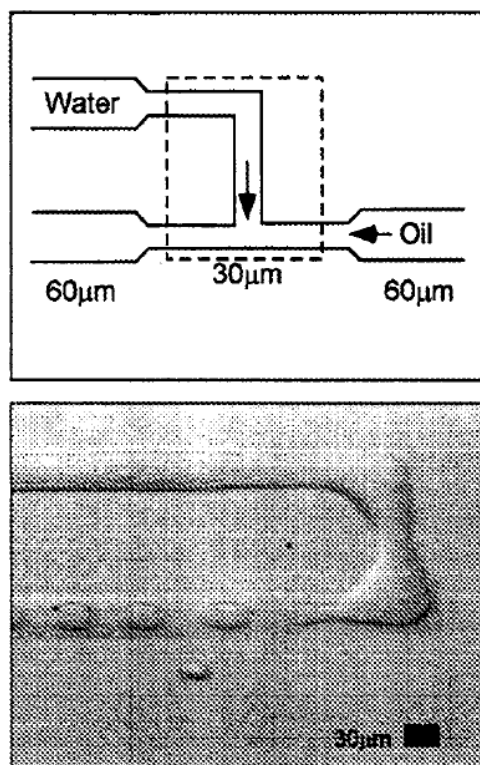


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

826. While it is my opinion that Thorsen discloses forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

827. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

828. It also would have been obvious to form at least one plug of the aqueous fluid

containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

829. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

830. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited

therein.

831. Claim 1 further recites: “**the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.**”

832. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

833. Figure 1, showing droplets surrounded by the oil, is reproduced below:

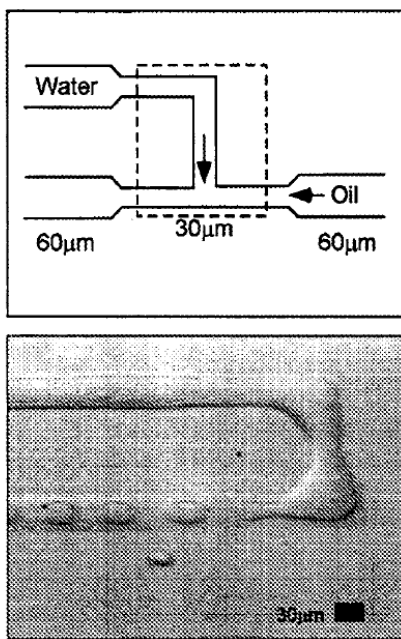


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

834. Claim 1 further recites: **“wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.”**

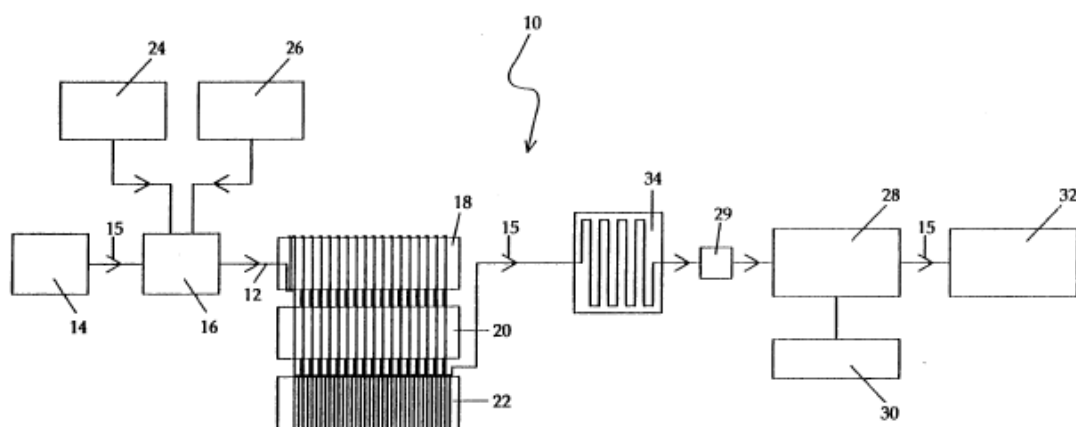
835. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

836. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

837. While it is my opinion that Thorsen discloses a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

838. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire

microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

839. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

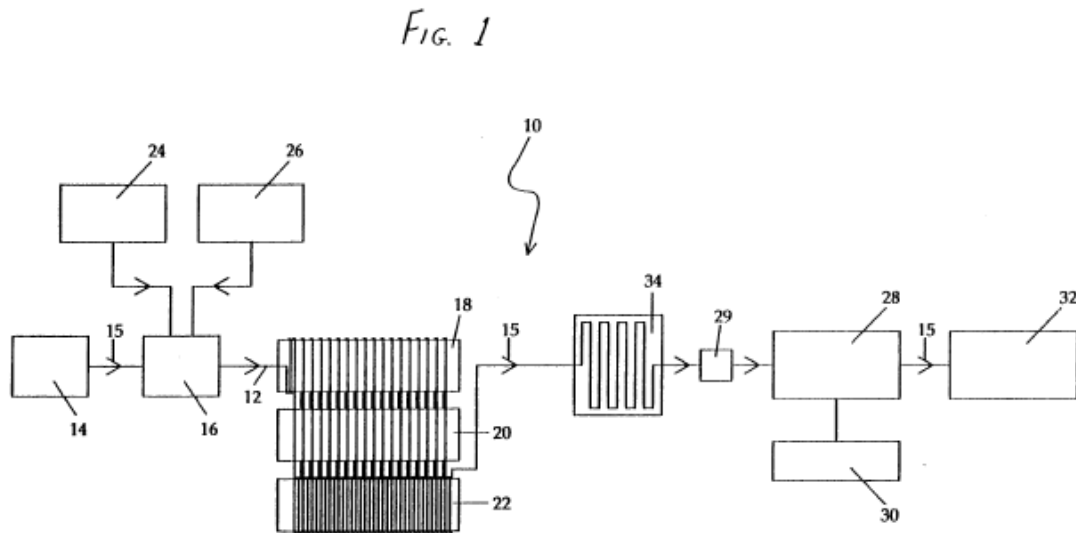
840. It also would have been obvious to form a plug comprising at least one biological

molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

841. Claim 1 further recites: “**and providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.**”

842. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable

components in a small volume, approximately 5-20 μl , is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

843. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μM thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

844. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

845. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all

references cited therein.

(ii) *Claim 2*

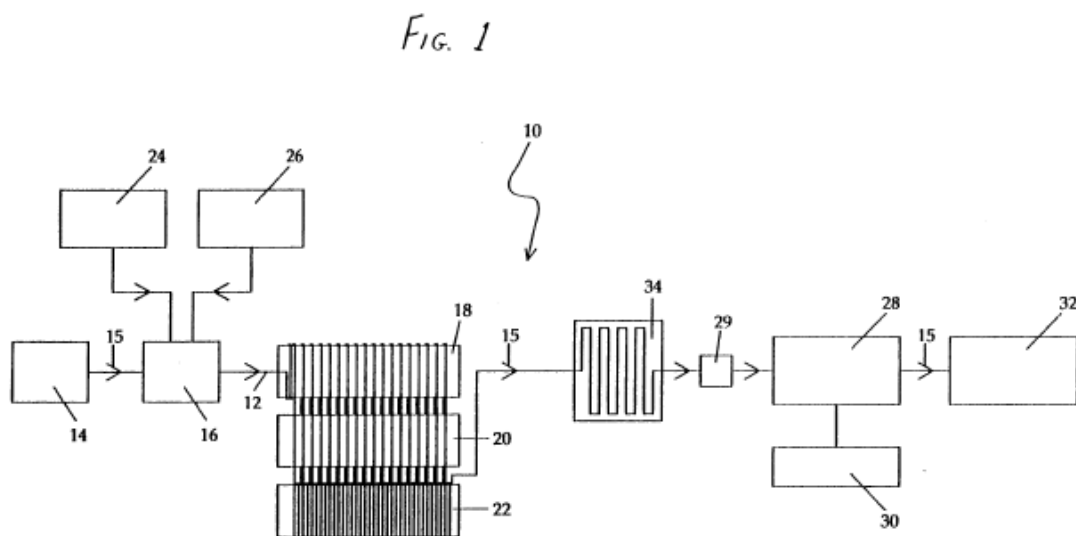
846. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

847. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

848. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to DNA or RNA.

849. While it is my opinion that Thorsen discloses that the at least one biological molecule is DNA or RNA, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA

sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

850. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose

(HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

851. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

852. I also incorporate by reference my opinions discussing the disclosure of this claim limitation in Quake. *See supra* ¶¶ 439-442. It would have been obvious to combine the teachings of Quake with the teachings of Thorsen.

853. It also would have been obvious that the at least one biological molecule is DNA or RNA based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited

therein.

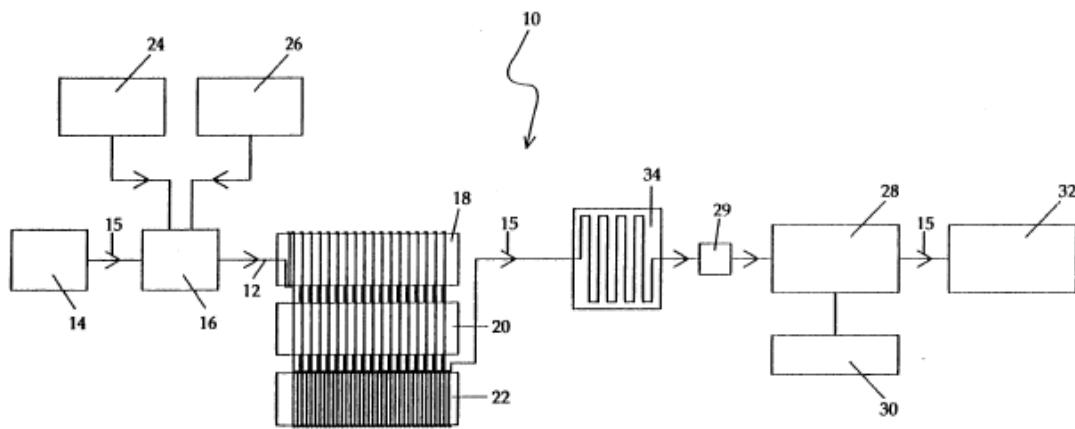
(iii) *Claim 3*

854. Claim 3 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

855. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

856. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

857. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

858. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

859. It also would have been obvious to conduct an autocatalytic reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

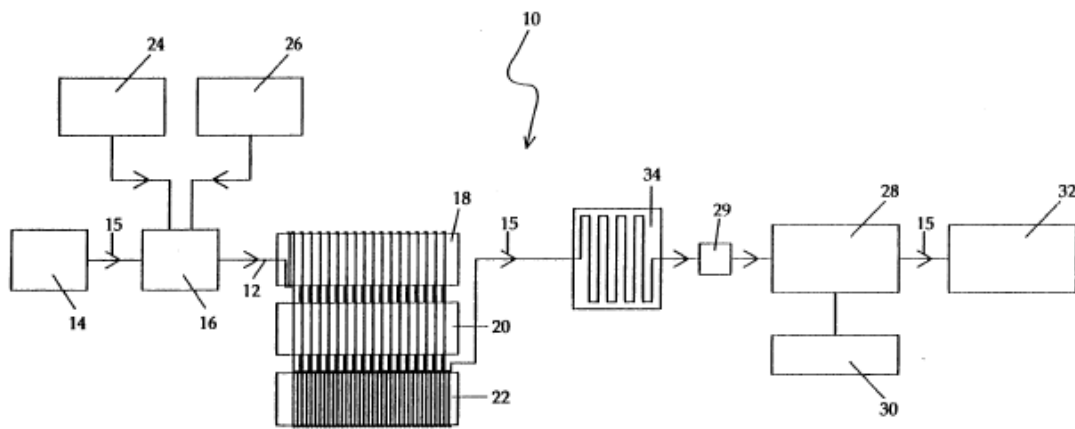
(iv) *Claim 4*

860. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

861. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

862. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

863. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

864. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

865. It also would have been obvious to conduct a polymerase-chain reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

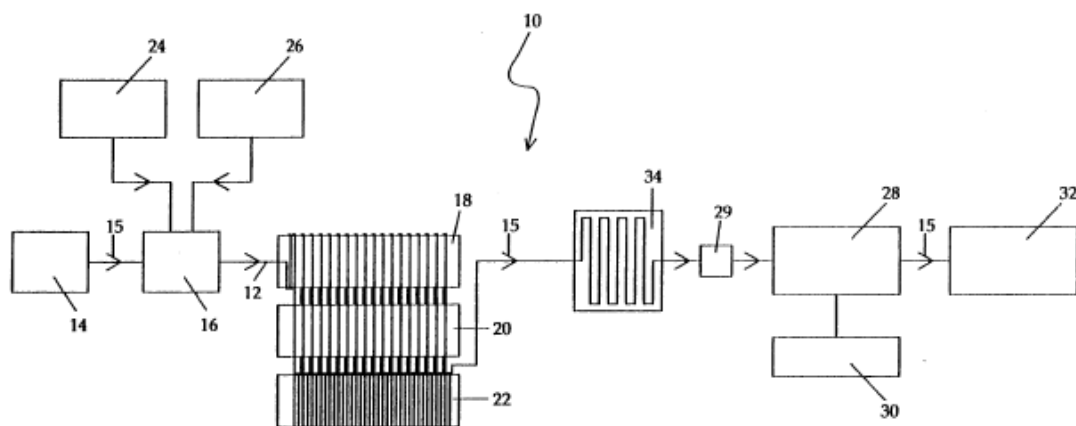
(v) *Claim 5*

866. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

867. Claim 5 further recites: **“the reaction is an enzymatic reaction.”**

868. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understand that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

869. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

870. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

871. It also would have been obvious to conduct an enzymatic reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

872. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

873. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

874. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

875. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

(vii) *Claim 9*

876. Claim 9 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

877. Claim 9 further recites: “**the oil comprises a surfactant.**”

878. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

879. Thorsen further describes that “[v]arious oils were tested in the device, including decane, tetradecane, and hexadecane, *combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.*” Thorsen at 4164 (emphasis added).

(viii) *Claim 10*

880. Claim 10 of the ’407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

881. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

882. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

883. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorosurfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

884. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

885. It also would have been obvious to use a fluorosurfactant based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

886. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference

my analysis with respect to claims 1 and 8.

887. Claim 11 further recites: “**the oil is a fluorinated oil.**”

888. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

889. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

890. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects.

Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

891. It also would have been obvious to use a fluorinated oil based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

892. Claim 13 of the ’407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

893. Claim 13 further recites: “**the providing step includes heating.**”

894. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

895. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

896. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

897. It also would have been obvious to provide heating to the microfluidic system based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(f) Invalidity Based on Seki

898. It is my opinion that Seki discloses and/or renders obvious all elements of claims 1-5, 8-11, and 13 of the '407 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

899. The preamble of claim 1 of the '407 patent recites: “**A method for conducting a reaction in plugs in a microfluidic system.**”

900. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

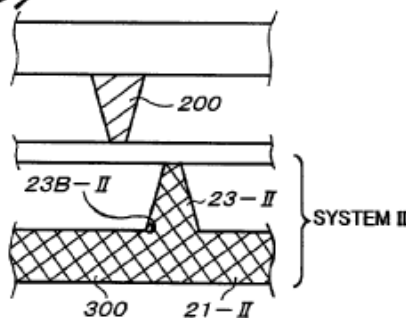
901. Regardless of whether the preamble is limiting, Seki satisfies this claim

limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

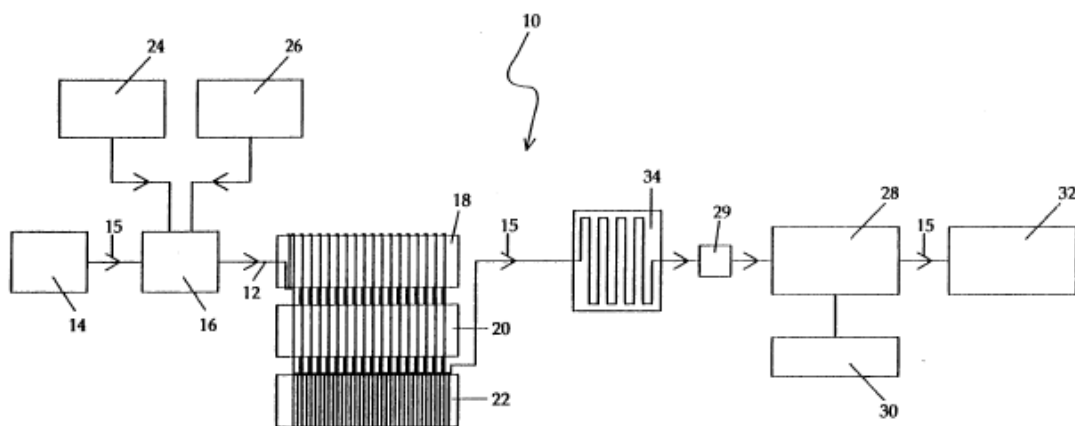
902. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:

FIG. 10(c)

Seki at Fig. 10(c).

903. While it is my opinion that Seki discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

904. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the

separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

905. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

906. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

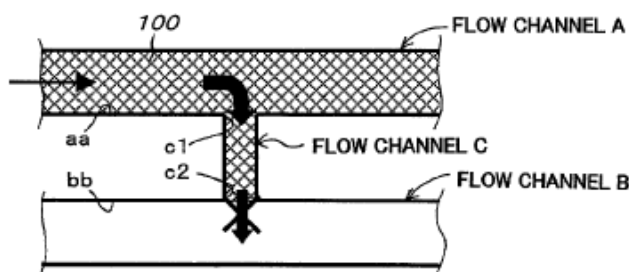
“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

907. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

908. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

909. Seki satisfies this limitation. For example, Seki describes a microfluidic system with at least two junctions having at least one junction:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.



Seki at Abstract.

910. Claim 1 further recites: **“continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels.”**

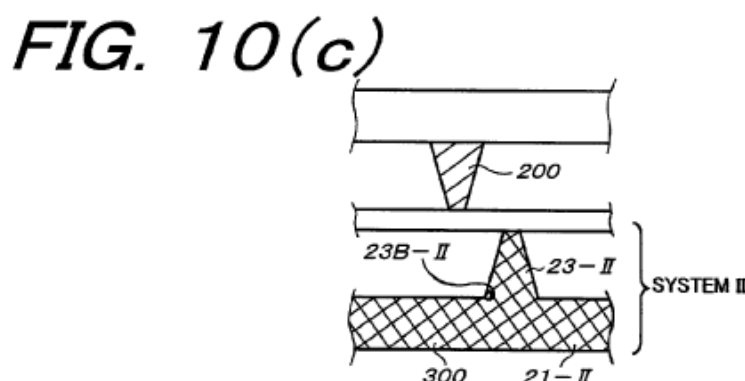
911. Seki discloses this limitation. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

912. Seki describes that a reaction can be conducted within a droplet. “In this case,

coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

913. Seki also discloses applications for his system, using biological molecules. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

914. While it is my opinion that Seki discloses flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for

use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

915. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

916. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological

molecule and the at least one reagent through a first channel of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

917. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

918. It also would have been obvious to flow an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

919. Claim 1 further recites: **“continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels.”**

920. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

921. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels.”**

922. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and

the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

923. While it is my opinion that Seki discloses forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

924. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire

microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

925. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

926. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

927. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

928. Claim 1 further recites: **“the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel.”**

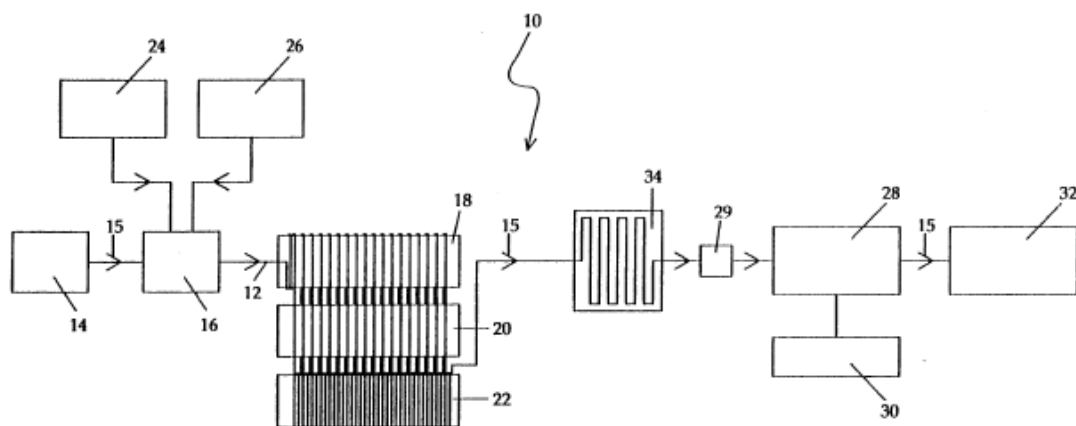
929. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

930. Claim 1 further recites: **“wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule.”**

931. Seki discloses this limitation. Seki describes droplets comprising at least one biological molecule and at least one reagent for conducting the reaction with the at least one biological molecule. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

932. While it is my opinion that Seki discloses a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

933. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

934. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

935. It also would have been obvious to form a plug comprising at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

936. Claim 1 further recites: “**and providing conditions suitable for the reaction in**

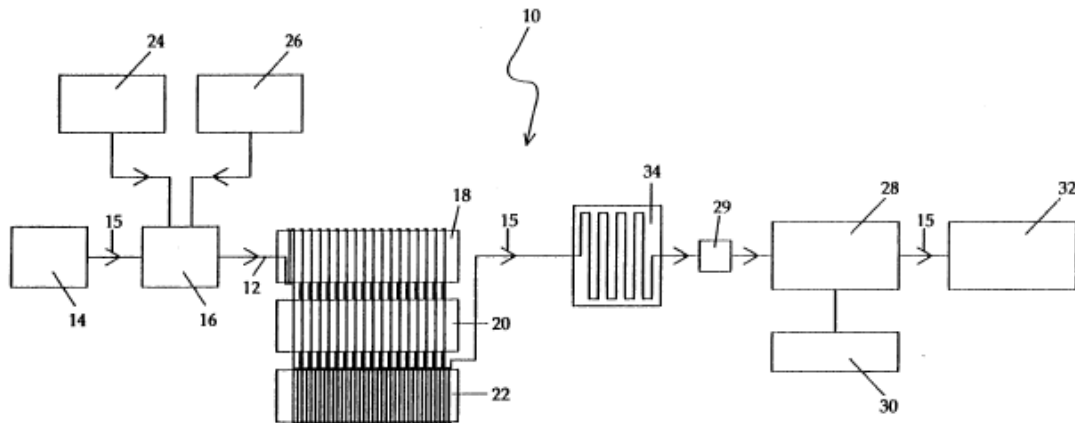
the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.”

937. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes the conditions suitable for conducting a glucose reaction: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

938. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is

reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

939. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were

clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

940. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

941. It also would have been obvious to provide conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

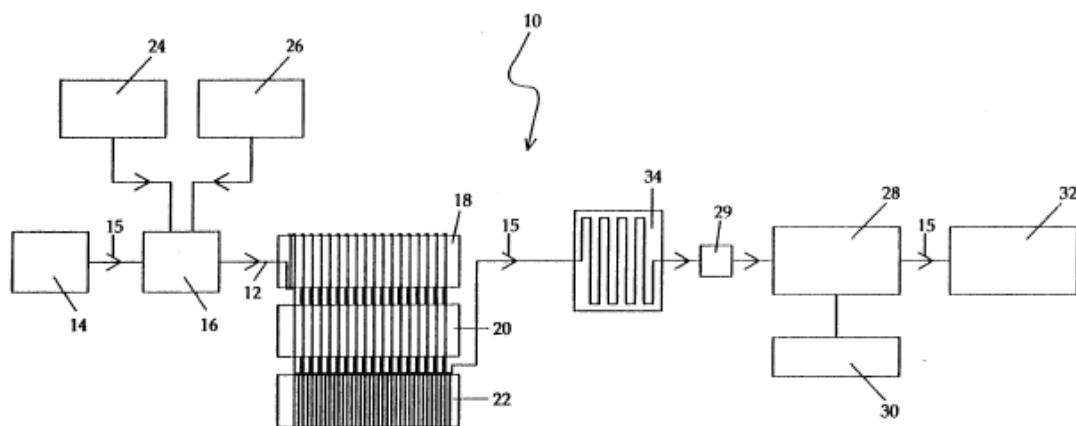
(ii) *Claim 2*

942. Claim 2 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

943. Claim 2 further recites: “**the at least one biological molecule is DNA or RNA.**”

944. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

945. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was

opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

946. It also would have been obvious that the at least one biological molecule is DNA or RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

947. I also incorporate by reference my opinions discussing the disclosure of this claim limitation in Quake. *See supra* ¶¶ 439-442. It would have been obvious to combine the teachings of Quake with the teachings of Seki.

948. It also would have been obvious that the at least one biological molecule is DNA or RNA based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

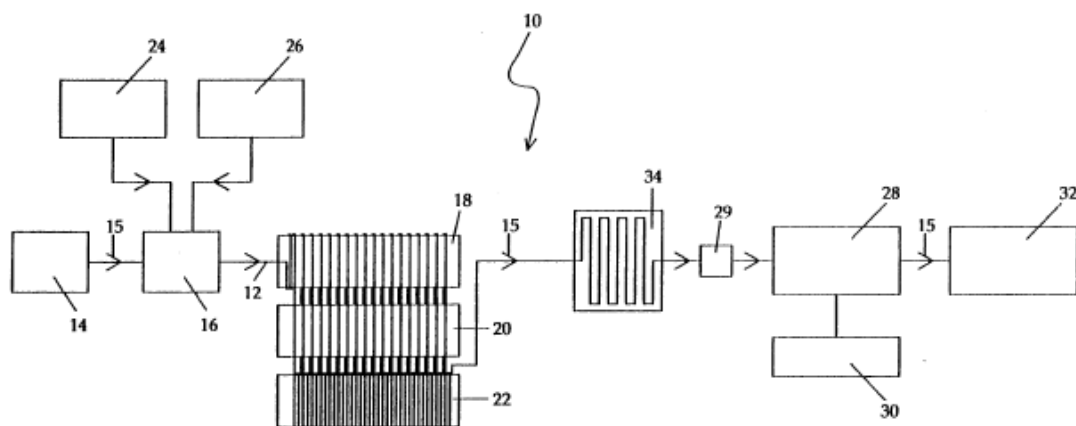
949. Claim 3 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

950. Claim 3 further recites: “**the reaction is an autocatalytic reaction.**”

951. Seki at least renders obvious this limitation, in light of the background knowledge

of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

952. It also would have been obvious to conduct an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

953. It also would have been obvious to conduct an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

954. It also would have been obvious to conduct an autocatalytic reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

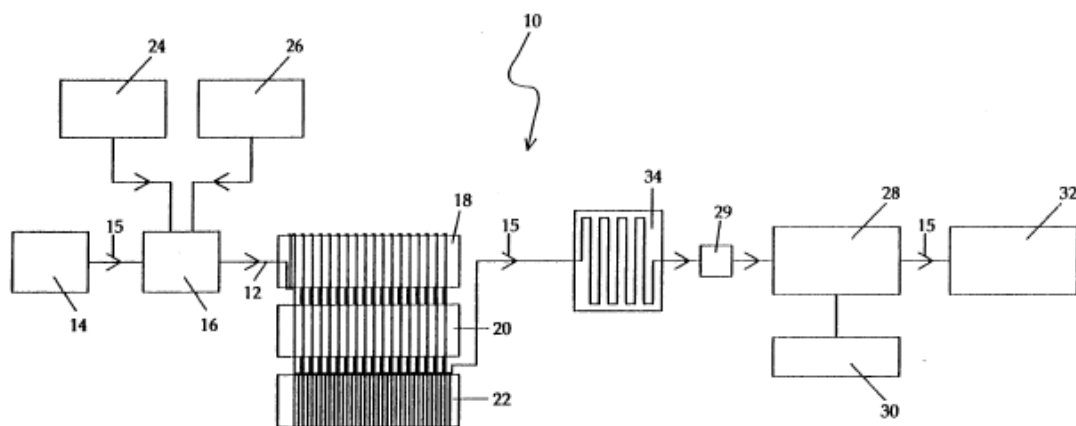
(iv) *Claim 4*

955. Claim 4 of the '407 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

956. Claim 4 further recites: “**the reaction is a polymerase chain reaction.**”

957. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

958. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

959. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

960. It also would have been obvious to conduct a polymerase-chain reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

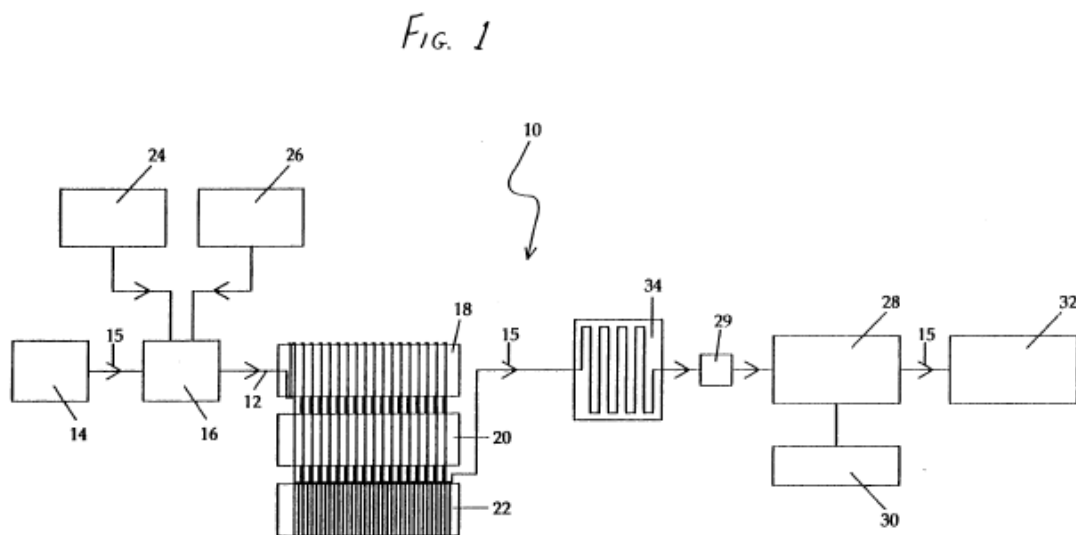
961. Claim 5 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

962. Claim 5 further recites: **“the reaction is an enzymatic reaction.”**

963. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes an enzymatic reaction using glucose as a reagent: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

964. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element, including references that discuss PCR. A POSA would have understood that PCR is an enzymatic reaction, because it uses a polymerase enzyme. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

965. It also would have been obvious to conduct an enzymatic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a

passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

966. It also would have been obvious to conduct an enzymatic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

967. It also would have been obvious to conduct an enzymatic reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

968. Claim 8 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

969. Claim 8 further recites: “**the immiscible carrier fluid is an oil.**”

970. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 467-471, demonstrating how Quake discloses an immiscible carrier fluid that is an oil.

971. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use an oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

972. It also would have been obvious to use an oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

973. It also would have been obvious to use an oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid

which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

974. It also would have been obvious to use an oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 9*

975. Claim 9 of the ’407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

976. Claim 9 further recites: “**the oil comprises a surfactant.**”

977. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

978. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that

these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

979. It also would have been obvious to use a surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 10*

980. Claim 10 of the '407 patent is dependent on claim 9. I incorporate by reference my analysis with respect to claims 1, 8, and 9.

981. Claim 10 further recites: “**the surfactant is a fluorosurfactant.**”

982. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorosurfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

983. It also would have been obvious to use a fluorosurfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

984. It also would have been obvious to use a fluorosurfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

985. Claim 11 of the '407 patent is dependent on claim 8. I incorporate by reference my analysis with respect to claims 1 and 8.

986. Claim 11 further recites: **“the oil is a fluorinated oil.”**

987. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

988. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

989. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or

manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

990. It also would have been obvious to use a fluorinated oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 13*

991. Claim 13 of the '407 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

992. Claim 13 further recites: “**the providing step includes heating.**”

993. Seki satisfies this limitation. For example, Seki discloses that “[a] temperature of the microchip is adapted to be controlled by a temperature controller.” Seki at [0126].

994. While it is my opinion that Seki discloses a method for conducting a reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to provide heating to the microfluidic system in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the

amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

995. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

996. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

997. It also would have been obvious to provide heating to the microfluidic system based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and IX.D.3 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3. *Motivation to Combine and Reasonable Expectation of Success*

998. A POSA would have seen compelling reasons to modify the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct different

types of reactions (and in particular, PCR) in small volumes as taught by Corbett, Lagally, Burns (1996), or Wang. This is because the prior art clearly taught that reactions could be conducted within microfluidic droplets, and there were numerous advantages associated with these microfluidic droplet reactors. In particular, a POSA would have considered it obvious to modify the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR based on numerous teachings in the art, including Corbett, Lagally, and Burns (1996), which discussed small-scale and even on-chip PCR (*see, e.g.*, Burns (1996)). A POSA would have had a reasonable expectation of success in so modifying, as evidenced by both the prior art and contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. Indeed, Quake itself describes both enzymatic reactions with biological molecules and PCR within microfluidic droplets. Quake at [0080] and [0170].

999. A POSA would have been strongly motivated to perform reactions with biological molecules, including PCR, in the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki because doing so would have provided the substantial benefits known to be associated with microfluidic reactors. For example, Nisisako noted that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation, and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” *Treating liquid samples in droplet shape has the advantage that dead volume can be decreased.* Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is *likely to become increasingly important.*” Nisisako at 24 (emphasis added).

1000. A POSA would also prefer to carry out reactions in microfluidic droplets because its small dimensions allow for reduction of diffusion time for bimolecular reactions. Biomolecular reactions require two molecules to first encounter each other by diffusion or convection-enhanced diffusion. The reaction time and reaction yield for a given reactor are then determined by the diffusion time and then the kinetic time after the molecular encounter. By reducing the diffusion time, a micro-droplet reactor can significantly enhance the reaction yield. *See, e.g.,* Burns (2001) at 10. The reduction of the diffusion time also allows for careful analyses of different kinetic times or kinetic rates, thus allowing for the selection or screening of chemical or biological catalysts. If such reactions involve thermal programming, the low thermal capacitance of droplets also allows very rapid temperature change, thus preventing undesirable by-products.

1001. For example, with protein crystallization, the reduction of diffusion time reduces exposure to non-ideal environments during the random-walk diffusion *See, e.g.,* Chayen, N., et al., “Microbatch crystallization under oil – a new technique allowing many small-volume crystallization trials,” *Journal of Crystal Growth*, 122: 176-180 (1992) (“Chayen”) (10X-000254982-6). A large number of small-volume droplets can also enhance selectivity. If the concentration of the droplets is higher than the concentration of interfering agents, the concentration of the interfering agents will be lower in the droplets. *See, e.g.,* Ferrance at 200.

1002. As another example, Lagally explained that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to *increase the speed of these assays* and to *reduce the amount of material and reagents needed.*” Lagally at 565 (emphasis added). Because PCR and other reactions with biological molecules rely on reagents that are often in limited supply—for example, sample

DNA—the ability to reduce both the amount of material needed for the reaction to occur and the dead volume of the reaction would have been highly motivating. Ferrance similarly explained that “[t]he same advantages of *reduced time, sample, and reagents* brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions.” Ferrance at 192 (emphasis added). The modification of Corbett, Lagally, Burns (1996), or Wang to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn would decrease operating costs. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

1003. Curcio likewise taught that “[m]initurization of the fluidic system is beneficial in two ways: it *enhances the speed of thermal equilibration of the reaction mixture*, thus allowing increased flow velocities and faster PCR. Also *analyte volumes are reduced*, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level.” Curcio at 7 (emphasis added).

1004. Vogelstein additionally taught that microfluidic PCR enabled a sample to be diluted into thousands of discrete reaction volumes that each contained either one template PCR molecule or no DNA molecules. Vogelstein at 9236, 9239. A POSA would have found this advantageous because individual-template PCR reactions would have enabled the detection of relatively rare mutations, dislocations, and allelic imbalances. Vogelstein at 9236, 9239.

1005. Reduction in size of the reaction vessel also allows for precise quantification of,

for example, nucleic acids and pathogens. As a single template nucleic acid or pathogen can be placed in a droplet, detection of successful PCR amplification in a given number of droplets allows for digital quantification of, for example, the number of template nucleic acid or pathogens present. For the same reasons, other types of patterns, including *irregular* expression of nucleic acids, could also be quantified. A POSA would have expected that the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform different types of reactions with biological molecules, including PCR, that would enable these applications.

1006. Further, conducting PCR in microfluidic droplets would reduce potential contamination of the reaction, an issue that the prior art had recognized. *See, e.g.*, Corbett at 3:6-12 (“The most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of the assay.”).

1007. It was also well known that decreasing the scale of reactions with biological molecules, including PCR, to microfluidic levels provided the substantial advantage of making reactors portable. For example, Kopp explained that portable PCR microreactors could enable “[o]n-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Kopp at [1047]. Further, it was known that portable PCR reactors could aid physicians in the development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Kopp at [1047]. Thus, the prior art demonstrated that using the droplet reactors of Quake, Shaw Stewart, Burns (2001),

Nisisako, Thorsen, or Seki to perform PCR (and other reactions with biological molecules) would have advantageously allowed PCR and other biological reactions to be performed in point of care diagnostic applications.

1008. Additionally, using the microfluidic reactors for PCR reactions would have substantially increased the tolerance of PCR reactions to primer non-specificity. As of the filing date, it was well known that PCR reactions suffered from the limitation that the primers were not always specific to the sequence of interest but rather could also bind to other sequences. *See, e.g.,* Cha et al., Specificity, Efficiency, and Fidelity of PCR, *PCR Methods and Applications*, 3:518-529 (1993) (“Cha”) (10X-000255918-30) at 526. Because PCR amplification reactions are exponential in nature, PCR would often be ineffective where these other DNA fragments outnumbered the fragments of interest. *Id.* In such circumstances, the amplification products of the former would greatly exceed the amplification of the latter. *Id.* By using multiple droplets, a POSA could reduce the chances of having an uncontaminated DNA template in a single reaction. *Id.* Further, a POSA could conduct exponential amplification of the template without having the intended amplification product compete with unintended amplification products. *Id.*

1009. Moreover, a POSA would have expected the combination of microfluidic droplet reactors and different types of biological reactions, including PCR, to be successful. For example, in 2001, Lagally et al. provided an overview of the evolution of continuous flow PCR microreactors:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR

reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Lagally at 565-566.

1010. In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct single-molecule DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA

molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

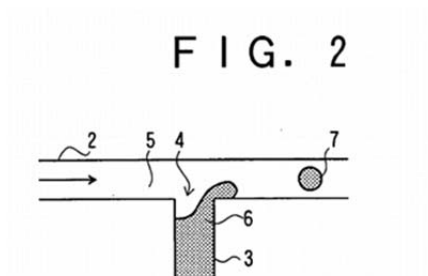
The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Lagally at 566-570. Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. Consistent with the observations in the prior art, a POSA would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR reactions or other reactions with biological molecules.

1011. The fact that several other groups simultaneously developed microfluidic systems that fall within the claims of the Ismagilov patents provides further evidence that a POSA would have both found the combinations described above obvious and would have had a reasonable

expectation of success in so combining. For example, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001, more than a year prior to the '407 patent's earliest claimed priority date. *See* Higuchi I; Japanese Application No. 2001-048097 ("Higuchi II") (10X-000255281-307); and Japanese Application No. 2001-238624 ("Higuchi III") (10X-000255308-47).

1012. Higuchi I discloses "a process and apparatus for rapidly producing an emulsion and microcapsules in a simple manner." Higuchi I at Abstract. As an example, Higuchi describes that "[a] process for producing an emulsion includes a step of ejecting a dispersion phase from a dispersion phase-feeding port toward a continuous phase flowing in a microchannel in such a manner that flows of the dispersion phase and the continuous phase cross each other, whereby microdroplets are formed by the shear force of the continuous phase and the size of the microdroplets is controlled." Higuchi I at [0006]. This is illustrated by Figure 2 in Higuchi I, reproduced below:



Higuchi I at Fig. 2. In the text accompanying the figure, and corresponding with the numbers, Higuchi I describes that "[a] dispersion phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Abstract. The

microfluidic droplet system Higuchi and his colleagues developed was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet systems to create droplets from continuously flowing streams of water and oil—and the use of those droplets to conduct reactions—was within the level of skill in the art as of the earliest effective priority date.

1013. As another example, Todd Thorsen (who co-authored the Thorsen reference discussed above) also developed a droplet reactor that falls within the claims of the Ismagilov patents. Thorsen Thesis at 94-108. The Thorsen Thesis describes the following microfluidic droplet reactor:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

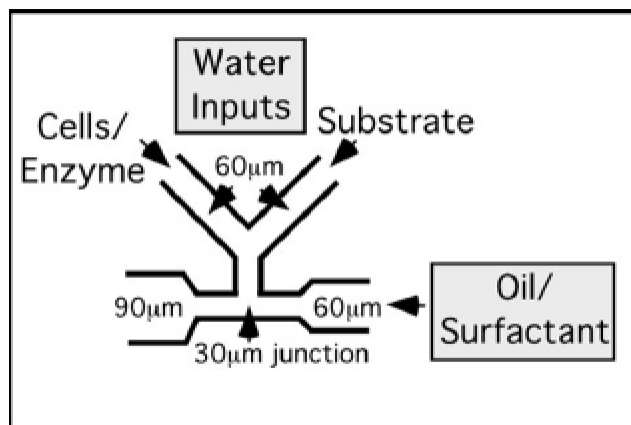


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

1014. The Thorsen thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002, suggesting that Thorsen’s work was performed before this date. The Thorsen Thesis was deposited with CalTech THESIS on December 2, 2002. Thorsen Thesis at 10X-000255686. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

1015. A POSA would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Schubert, or Krafft in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ramsey at 6:49-50 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). Krafft also noted that “the relatively low reactivity of fluorchemicals allows them to be combined with a wide variety of compounds without altering the properties of the incorporated agent.” Krafft at 2:27-30. For example, Curcio

described that perfluorodecalin was utilized as a carrier fluid with small-volume PCR because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity [and thus, high biocompatibility] towards biomolecules.” Curcio at 9. Therefore, a POSA conducting a reaction with a biological molecule in microfluidic droplets, such as PCR, would have used fluorinated oils and fluorinated surfactants with these microfluidic droplet systems. For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

1016. Further, fluorinated oil offers high immiscibility with water and low solubility of biomolecules. *See, e.g.*, Schubert at 97 (“Fluorinated compounds also offer the potential for biomedical applications. For example, . . . fluorinated alkanes are . . . chemically and biologically stable.”); *id.* (“Because fluorocarbons are insoluble in water, however, they are currently administered in the form of emulsions, the formation of which requires the use of surfactants.”). Unlike most mineral oils, fluorinated oil has a density higher than water. Gelest, Inc., *Silicone Fluids: Stable, Inert Media* (1998) (“Gelest”) (10X-000255623-49) at 19. This higher density allows easy separation of aqueous droplets from the oil when the emulsion is collected off the substrate.

1017. The art had also already noted that fluorination was preferable for silicon-based microfluidic devices, which have a tendency to swell when exposed to hydrocarbon oils. *See* Quake at [0118] (emphasis added) (“***TEFLON [which contains fluorination] is particularly suitable for silicon elastomer (RTV) channels***, which are hydrophobic and advantageously do

not absorb water, but *they may tend to swell when exposed to an oil phase.*”). As Quake noted, “[s]welling may alter channel dimensions and shape, and may even close off channels, or may affect the integrity of the chip, for example, by stressing the seal between the elastomer and a coverslip.” Quake at [0118]. This issue was also prevalent with PDMS, a silicon material that was commonly used to manufacture microfluidic substrates. *See* Quake at [0216] (emphasis added) (“In a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicon rubber or PolyDiMethylSiloxane (PDMS), *preferably PDMS.*”); ’407 patent at 16:59-61 (“Channels may be molded onto optically transparent silicon rubber or polydimethylsiloxane (PDMS), *preferably PDMS.*”).²⁰ Unlike other organic oils, fluorinated oil does not cause polymer like PDMS to swell. Holtze at 1632 (“In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.”) (citing Lee, J., et al., “Solvent Compatibility of Poly(dimethylsiloxane)-Based Microfluidic Devices,” *Anal. Chem.* 75:6544-6554 (2003)) (“Lee”) (10X-000255376-86). Therefore, a POSA would have been motivated to use fluorinated oils and surfactants to prevent swelling of the polymer substrate.

1018. Importantly, fluorinated oil is far less viscous than other oils, including mineral oils. *See generally* Gelest. Instead, fluorinated oil has a viscosity similar to water. *Id.* Using a fluorinated oil with a microfluidic droplet device would thus allow high-frequency generation of droplets and parallel generation with multiple orifices. The prior art had already shown that high-throughput droplet generation was desirable. *See* Quake at [0079] (“This arrangement can be used to improve throughput or for successive sample enrichment, and can be adapted to provide

²⁰ I note that this language in the Ismagilov patents was copied almost directly from Quake.

a very high throughput to the microfluidic devices that exceeds the capacity permitted by conventional flow sorters.”); Quake at [0093] (“Monodisperse droplets may be particularly preferabl[e], e.g., in high throughput devices and other embodiments where it is desirable to generate droplets at high frequency.”). Further, the viscosity of fluorinated oil is insensitive to temperature, which is particular useful for DNA amplification reactions involving temperature changes. This of course includes PCR. Mullis at 9:55-60. For these reasons, a POSA would have been motivated to use fluorinated oil to achieve higher frequency droplet generation. Indeed, fluorinated oil has become the preferred carrier fluid for high-throughput aqueous droplet microfluidics. Autour, A. and Ryckelynck, M., “Ultrahigh-throughput improvement and discovery of enzymes using droplet based microfluidic screening,” *Micromachines*, 8:128(2017) (“Autour”) (10X-000254961-81) at Section 4.1.

1019. As the prior art demonstrates, a POSA would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions, including PCR, as taught by Corbett, Lagally, Burns (1996), or Wang.

X. INVALIDITY OF THE '193 PATENT

A. Summary of the '193 Patent

1020. The '193 patent is entitled "Method for Conducting an Autocatalytic Reaction in Plugs in a Microfluidic System." The abstract explains that the invention "provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid." '193 patent at Abstract.

1021. I understand that Bio Rad is asserting claims 1-8 and 11 of the '193 patent. Of these claims, only claim 1 is independent. Claims 2, 4-7, and 11 depend on claim 1. Claim 3 depends on claim 2 and claim 8 depends on claim 7.

1022. The '193 patent issued from Application No. 13/024,155, filed July 21, 2011 (the "'155 application"). The '155 application was a continuation of application No. 12/777,099, filed on May 10, 2010, which was a continuation of application No. 10/765,718, filed on January 26, 2004, which itself was a continuation-in-part of application No. 10/434,970 (which issued as the '091 patent), filed on May 9, 2003.

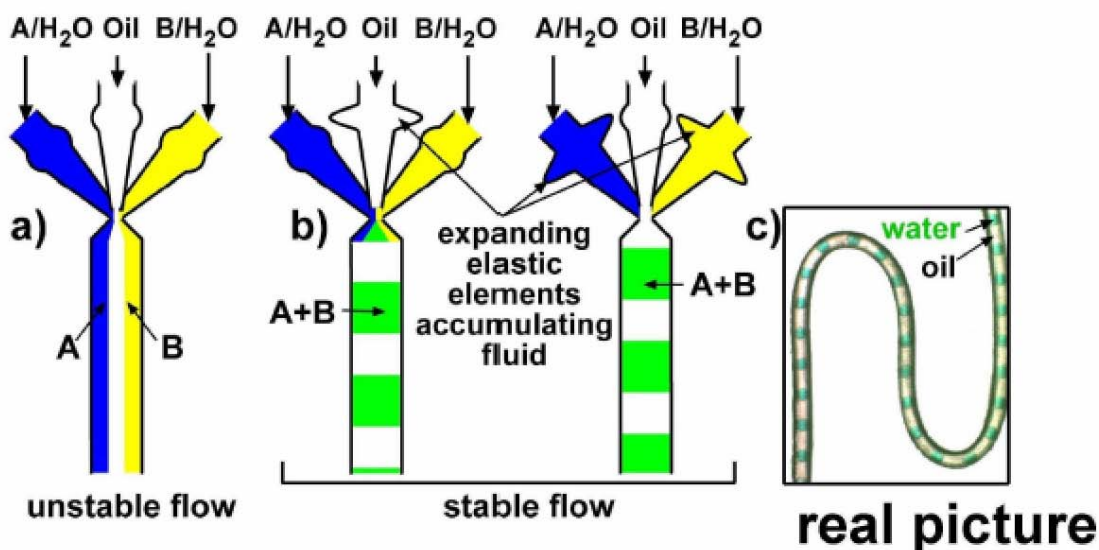
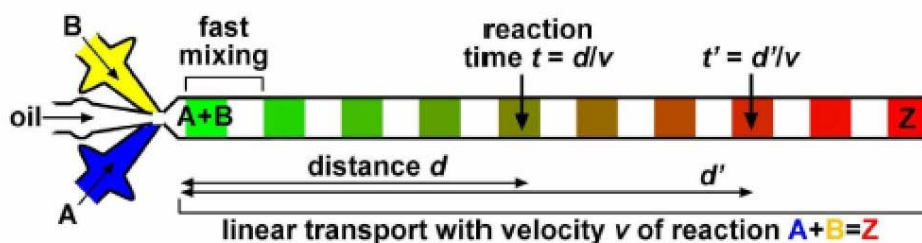
1. Priority

1023. I understand that Bio-Rad asserts that claims 1-2, 4-5, 7, and 11 were conceived of "no later than October 16, 2001," and relies on RI00111660-70 to support this assertion. Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 6. I further understand that Bio-Rad asserts that claim 3 was conceived of "no later than October 30, 2002" and relies on RI00111690-738 to support this assertion. *Id.* I also understand Bio-Rad asserts that claim 6 was conceived of "no later than February 4, 2002," relying on RI00106817-18 to support this assertion, and that claim 8 was conceived of "no later than February 8, 2002," relying on RI00111308 and RI00111321 to support this assertion. *Id.* I disagree with Bio-Rad's assertions. The cited documents do not demonstrate the inventors had

formed in their minds the definite and permanent idea of a complete and operative invention as of the dates alleged.

1024. RI00111660-70, which Bio-Rad relies on to evidence conception of claims 1-2, 4-5, 7, and 11, appears to be a portion of a PowerPoint presentation. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 16, 2001, but at best sets forth a research plan. As an initial matter, while the first page of the presentation is dated October 16, 2001, the document's metadata does not indicate when this PowerPoint was last modified or otherwise substantiate this date. Further, the document does not establish that the inventors had possession of every feature recited in claims 1-2, 4-5, 7, and 11 or that every limitation of the claim was known to the inventor as of this date. For example, each claim in the '193 patent requires a "plug" that it "substantially surrounded by an oil flowing through the channel," and this limitation is not suggested by any of the figures in RI00111660-70, which depict channels in with regions occupied by an "oil" and regions occupied by mixtures of aqueous fluids. The aqueous fluids do not appear to be substantially surrounded by oil. Instead, the aqueous fluid regions depicted appear to be "slugs:"

Solution - true plug flow?



See, e.g., RI00111663. In fact, the slide suggests expanding elements are necessary to achieve stable flow. Unstable flow results when aqueous streams contact the main channel, which is indicative of a hydrophilic channel. This would suggest that the aqueous plug fluid can be in contact with the channel wall without significant encapsulation by the carrier oil fluid. Further, the channel layout depicted in these slides is not appropriate for plug formation. Instead of the T-junction approach later used in the Ismagilov patents, these slides depict a single oil channel between two aqueous channels. These channels do not intersect the main channel at an angle. This channel layout is also not appropriate for flow focusing as the two aqueous channels surround a single oil channel. The inverse would be used for flow focusing (two oil channels

surrounding a single aqueous channel). Based on my experience, this channel layout would not enable plug formation.

1025. As another example, each claim of the '193 patent requires "flowing an aqueous fluid" and "flowing an oil" to form "at least one plug." These limitations are not suggested by any of the slides in RI00111660–70, which do not depict or describe the formation of plugs from a "flowing aqueous fluid" and "flowing oil." Instead, as discussed above, the slides appear to depict "slugs" and the slides do not provide any indication that the aqueous fluid and oil are "flowing."

1026. As another example, claim 4 recites the method according to claim 1, in which one step "includes heating," but there is no depiction or description of heating in RI00111660–70.

1027. As another example, claim 7 recites the method of claim 1 "wherein the carrier fluid comprises a surfactant," but this limitation is not suggested by any of the slides in RI00111660–70, which do not depict or describe any surfactant.

1028. Similarly, for example, claim 11 recites "[t]he method of claim 1, wherein the at least one plug is substantially spherical in shape," and this limitation is nowhere suggested in RI00111660–70, which nowhere depicts or describes a plug that is "substantially spherical" in shape.

1029. RI00111690–1738, which Bio-Rad relies on to evidence conception of claim 3, appears to be a grant application that was signed on October 30, 2002. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 30, 2002, or establish that the inventors had possession of every feature recited in claim 3 as of that time. For example, claim 3 depends from

claim 1, which requires “at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule,” and additionally adds the limitations that “the at least one substrate molecule is a single biological molecule” and that “the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” RI00111690–1738, however, does not discuss providing conditions suitable for a polymerase chain reaction involving a single DNA molecule. The only specific reference to DNA or RNA in RI00111690-1738 is the statement that “schemes can be readily constructed in which a single molecule of DNA, RNA, or a protein labeled with nanoparticles is detected visually via [an] autocatalytic pathway” after the molecule is “label[ed]” with metallic autocatalyst—*not* after that molecule undergoes a polymerase chain reaction. RI00111722–23. Similarly, the only discussion of any “polymerase chain reaction” in the cited application is as a general example (along with silver halide photography) of a context in which autocatalytic reactions may take place. RI00111719.

1030. RI00106817-18, which Bio-Rad relies on to evidence the conception of claim 6, appears to be two lab notebook entries. This document does not demonstrate that the inventors had formed in their minds the definite and permanent idea of a complete and operative invention as February 4, 2002. As an initial matter, while the first entry bears the date February 4, 2002, the second entry does not, and Bio-Rad has identified no evidence corroborating its apparent assertion that either of these entries were created on February 4, 2002 (for example, these notes were not witnessed or countersigned by a third party). Further, the document does not establish that the inventors had possession of every feature recited in claim 6, or that every limitation of the claim was known to the inventor as of the date of the entry. For example, claim 6 depends from claim 1, which refers to “reagents for conducting an autocatalytic reaction.” RI00106817

contains no references to any reactions, autocatalytic or otherwise. Instead, RI00106817 appears to refer to an experiment where streams of water (with dye) were introduced along with perfluorodecalin into a microchannel, with the observed result that “water and oil plugs were found to form consistently.” The reference to an apparently subsequent experiment involving “Fluorescence testing” in RI00106818 dated February 5, 2002 and contains no references to plugs, microchannels, or providing conditions suitable for autocatalytic reactions in a microfluidic system.

1031. RI00111308 and RI00111321, which Bio-Rad relies on to evidence conception of claim 8, also appear to be entries in a lab notebook. These documents do not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as February 8, 2002, or establish that the inventors had possession of every feature recited in claim 8 as of that time. Bio-Rad has identified no evidence corroborating its apparent assertion that either of these entries were created on or before February 8, 2002 other than the dates written on these notebook pages (for example, these notes were not witnessed or countersigned by a third party), and one of these entries, RI00111321, bears a date (“3/10/2002”) after February 8, 2002.

1032. I understand that Bio-Rad asserts that each of claims 1-2, 4-8, and 11 in the ’193 patent was reduced to practice by May 9, 2002, based on the filing of the Ismagilov ’927 provisional application on that date. *See* Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 6. I further understand that Bio-Rad asserts that claim 3 of the ’193 patent was reduced to practice by May 9, 2003, based on the filing of the ’970 application on that date. *Id.*

1033. While I agree with Bio-Rad’s apparent admission that the ’927 provisional

application fails to contain sufficient written description to establish that the inventors had possession of the alleged invention of claim 3 of the '193 patent, and/or fails to enable claim 3 of the '193 patent, I also believe that the '927 provisional application does not contain sufficient written description to establish that the inventors had possession of any other alleged inventions of asserted claims in the '193 patent, and fails to enable any other asserted claims of the '193 patent. This is discussed in more detail below, for example, because all of the deficiencies identified in the specification of the '193 patent are also present in the '927 provisional application. Accordingly, the listed claims of the '193 patent are also not entitled to claim priority to the '970 application.

1034. I further note that the application that became the '193 patent was filed on February 9, 2011, as a continuation of U.S. Patent Application No. 12/777,099 (the "'099 application"), which was filed on May 10, 2010 and subsequently abandoned. I understand that during the prosecution of the '193 patent, the examiner and the applicant repeatedly discussed the priority to which draft claims in the application that became the '193 patent were entitled.

1035. On February 9, 2011, for example, the examiner rejected the applicant's claim of the benefit of the priority of the '099 application, noting that the pending claims—like the claims that ultimately issued in the '193 patent—recited reagents for conducting “an autocatalytic reaction.” The examiner noted that “[t]he only mention[] of [an] autocatalytic reaction” was found in a single paragraph of the '099 application—a paragraph that only generally discussed autocatalytic reactions as “present[ing] an exciting opportunity for highly sensitive detection of minute amounts of autocatalysts” *See* '193 Prosecution History, Office Action (dated July 5, 2011) (RDTX00001984-1991) at 2. Significantly, this paragraph does not appear in the '927 provisional application.

1036. In response to the examiner's arguments, the applicant stated in a conclusory fashion that the claims then before the examiner were "entitled to a priority date of May 9, 2002," but offered only what it characterized as "analysis . . . that shows that the claims are entitled to at least a priority date of January 26, 2004" in an attempt to avoid a prior art reference identified by the examiner. '193 Prosecution History, Amendment and Response (dated December 16, 2011) (RDTX00002108-2191) at 4. The only evidence cited by the applicant in support of the "autocatalytic reaction" and "polymerase chain reaction" limitations was, again, passages in the '099 application not present in the '927 provisional application. *See, e.g., id.* at 16-19, 71-72 (citing "Example 8" and "Example 9" in the '099 application); *see also id.* at 20 (citing the discussion of "the polymerase-chain reaction (PCR)" as "[a]nother example of an autocatalytic reaction" in the '099 application).

1037. The examiner responded by rejecting the applicants' claims to the priority of the '099 application, noting that the '099 application's "only detailed disclosure of [an] autocatalytic reaction concerns exclusively inorganic complex of Co^{2+} . . . for which the reaction involving only the traces of the compound is well analyzed in the prior art" '193 Prosecution History, Office Action (dated January 28, 2012) (RDTX00002196-2205) at 2-4. Again, this disclosure is not present in the '927 patent application.

1038. Although the file history of the '193 patent reflects subsequent amendments to draft claims and the examiner's ultimate allowance of those revised claims, it nowhere contains any finding by the examiner that any claim in the '193 patent is entitled to the priority date of the '927 patent, or any non-conclusory argument from the applicant that any claim in the '193 patent is actually entitled to that priority date.

1039. I understand that Bio-Rad has not provided evidence of the inventors' diligence in

reducing the alleged inventions of the '193 patent to practice after Bio-Rad's alleged dates of conception, and consequently that there is no evidence that any claim of the '193 patent would be entitled to priority as of Bio-Rad's alleged dates of conception, even if these dates were uncontested (which they are not). Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 6. While Bio-Rad cites a number of documents "*see also*" and "*see, e.g.*" many of these documents do not appear to relate to diligence and none establish diligence. Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 5. For example, RI00111541-57, RI00111558-70, and RI00111572-79 are undated. RI00111688-89, RI00111794-97, RI00111580-636, RI00111679-83, RI0011690-738, RI00111673-76, and RI00111739-793 are dated on or after the alleged date of reduction to practice for many of the asserted claims. RI00111677-78 appears to be an email from Heinrich Jaeger and Rustem Ismagilov dated April 24, 2002. The email speaks, in the future tense, of potential future projects that "use microfluidics." It does not describe work which had been or was currently being performed. RI00111684-87 appears to be an email chain between Rustem Ismagilov, Vince Turitto, and Connie Hall dated March 30, 2002 to April 24, 2002. This email chain again speaks of potential future work, not work that had been or was currently being performed. RI00111571 appears to be typed notes titled "Microfluidics Assessment 3/15/2002" that refers generally to "[f]orming plugs." While these notes appear to describe work that has been performed relating to "plugs" it is not clear when this work was performed and RI0011571 is nearly five months after the alleged conception date for many of the asserted claims. Further, as set forth in **Exhibit 2**, I have reviewed various lab notebooks from Dr. Ismagilov's lab dated before May 9, 2003. None of these notebooks suggest that any work was done to reduce the inventions claimed in the '193 patent to practice in the three months between the alleged date of

conception of claims 1-2, 4-5, 7, 11 (October 16, 2001) and January 16, 2002 (RI00106805). For example, none of these lab notebooks include experiments or work relating to conducting reactions in plugs before January 16, 2002. As another example, none of these notebooks suggest that any work was done to reduce the invention claimed in claim 3 of the '193 patent to practice in the nearly seven months between the alleged date of conception (October 30, 2002) and the alleged date of reduction to practice (May 9, 2003). For example, none of these notebooks include experiments or work relating to conducting PCR in plugs.

1040. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception, diligence, or reduction to practice (and I understand that 10X's position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

B. Invalidity Overview

1041. As shown in further detail below, my opinions regarding the '193 patent include the following:

- All asserted claims are invalid under Section 112 for lack of proper written description, lack of enablement, and/or indefiniteness.
- All asserted claims are obvious in light of Quake under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Shaw Stewart under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Burns (2001) under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Nisisako under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Thorsen under Section 103 (either alone or in combination with other references).

- All asserted claims are obvious in light of Seki under Section 103 (either alone or in combination with other references).

C. Invalidity Under 35 U.S.C. § 112

1042. As described in further detail below, it is my opinion that the asserted claims of the '193 patent are invalid under 35 U.S.C. § 112.

1. *Written Description*

1043. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '193 patent are invalid for lack of written description.

1044. The claims of '193 patent, for example, require an **"autocatalytic reaction."** I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Bio-Rad appears to be taking the position that the **"autocatalytic reaction"** is far broader than what was disclosed in the '193 patent. Based on Bio-Rad's 4(c) disclosures, Bio-Rad contends that 10X performs a "DNA amplification reaction" in its 10X GemCode™ platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,304,193 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 11, 60, 65, 67, 84-85, 92; *see also* Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4,

Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 8. I have reviewed the '193 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. The specification describes a single DNA amplification reaction: "Another

example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’193 patent at 44:58-61; *see also* ’193 patent at 2:65-67. There is no mention in the ’193 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. Indeed, there is no indication that the inventors of the ’193 patent contemplated any DNA amplification reaction beyond the basic (and well-known) PCR reaction. Nor has Bio-Rad identified any disclosure in the ’193 patent specification that discloses other DNA amplification reactions.

1045. There is also, for example, no adequate description of performing a “**autocatalytic reaction**” in plugs *outside of a substrate*, including, for example, a DNA amplification reaction outside of a substrate. I have reviewed the ’193 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. There is no indication that the inventors of the ’193 patent contemplated performing a DNA amplification reaction in plugs off the substrate. Nor has Bio-Rad identified any disclosure in the ’193 patent specification that discloses a DNA amplification reaction in plugs off the substrate.

1046. Bio-Rad has taken the following position:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and]

all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X's Interrogatory No. 3. As an initial matter, none of the identified reactions are DNA amplification reactions.

1047. Further, in each of the “embodiments in which droplets are captured in a capillary tube” (the “capillary tube embodiments”) identified by Bio-Rad, the droplets remain separated by carrier-fluid such that the risk of droplet coalescence is minimized.

1048. First, Bio-Rad cites the description of “a microfluidic device of the present system can further include capillary tubing suitable for collecting plugs (‘the capillary device’; FIG. 46). . . . [where] [u]pon formation of plugs in the PDMS portion and their transfer into capillary tubing, the flow rates are stopped, the capillary tubing is disconnected from the PDMS portion and the ends are sealed by capillary wax.” ’193 patent at 58:47-49, 58:65-59:1. As shown in Figure 46, this “capillary device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

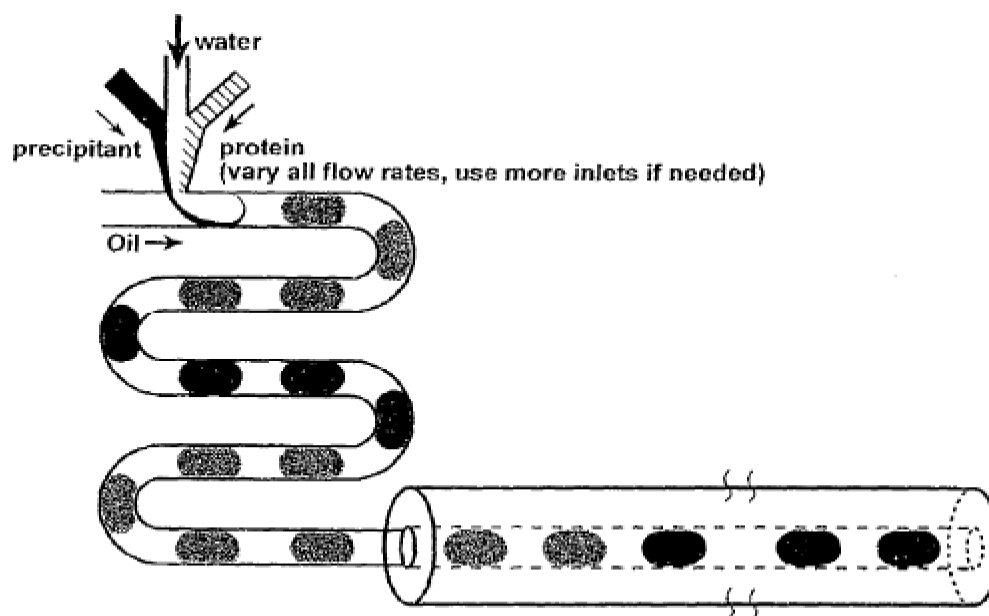


FIGURE 46

1049. Second, Bio-Rad cites Example 18 which states: “The capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator (18° C.). A lysozyme crystal appeared within an hour and was stable for at least 14 days without change of size or shape (FIG. 47A).” ’193 patent at 75:48-52. As shown in Figure 47A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

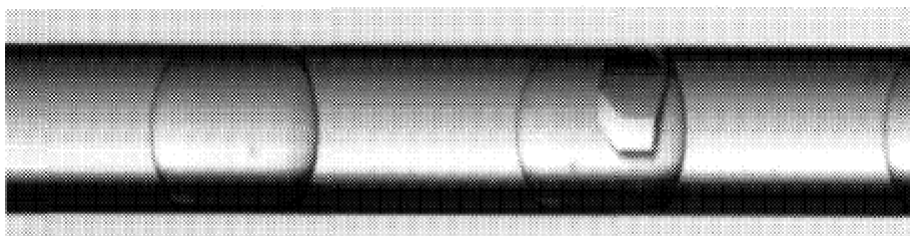


FIGURE 47A

1050. Third, Bio-Rad cites Example 19 which states: “The capillary was cut from the PDMS device, sealed by wax and stored in an incubator (18° C.). The thaumatin crystal appeared in 2-3 days and was stable for at least 45 days without size or shape change (FIG. 47B).” ’193 patent at 76:5-8. As shown in Figure 47B, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

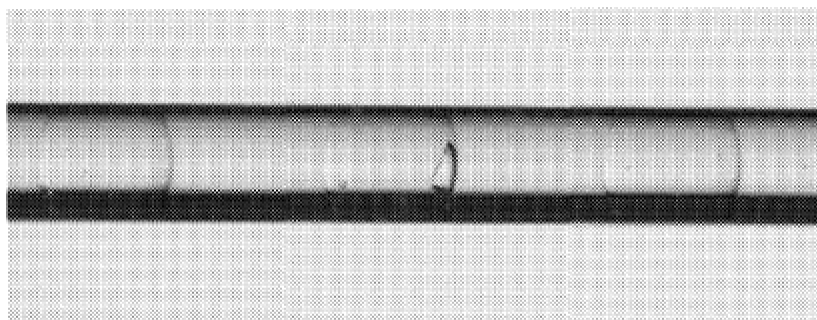


FIGURE 47B

1051. Fourth, Bio-Rad cites Example 20 which states in reference to Figure 50A:

“After establishing alternating aqueous droplet streams in the capillary, the flows were stopped, and the capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator at 18° C.” ’193 patent at 76:53-56. As shown in Figure 50A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

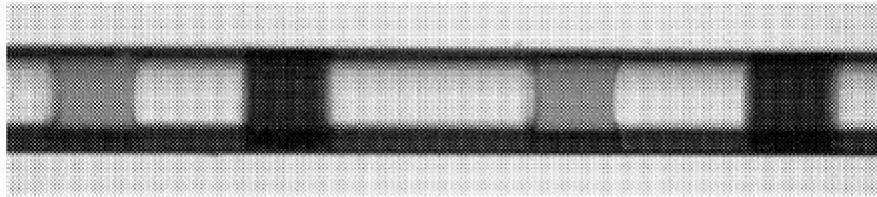


FIGURE 50A

1052. The “capillary tube embodiments” generally describe an extension of the microchannel that can be removed from the substrate while maintaining plug separation. These embodiments would not convey to a POSA that the inventors contemplated performing reactions in plugs outside of the substrate, for example in a well as performed by 10X. I understand that the image below is an image of droplets,

1053.

Due to a

difference in oil and water density, oil will drain out of the emulsion such that the droplets are closer together, increasing the potential of coalescence. The same is true for droplets collected using a micropipette or centrifuge tube. The capillary tube embodiments would not convey to a POSA that the investors had contemplated collection of droplets under these conditions. Further, as discussed below, Bio-Rad has not identified any teaching in the '193 patent that would convey to a POSA that the inventors had possession of a surfactant that would stabilize droplets and prevent droplet coalescence to allow for an "autocatalytic reaction" in plugs outside of the substrate, let alone a DNA amplification reaction in plugs outside of the substrate.

1054. In addition to the "capillary tube embodiments," Bio-Rad has cites to a portion of the specification as "*contemplat[ing]* collection of droplets and removal from the chip," First Supplemental Response to 10X's Interrogatory No. 3 (emphasis added):

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'193 patent at 17:16-23 (emphasis added). As an initial matter, this section "contemplates" collecting "*solution*" not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in "a standard 1.5 ml centrifuge tube" or the "[c]ollection . . . using micropipettes"²¹ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection, and subsequent

²¹ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 ("Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes."); *see also* Quake at [0148].

DNA amplification outside of the substrate.

1055. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. However, as discussed below, the surfactant depicted in Figure 24 would not stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets outside of the substrate.

1056. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927,²² these applications lack adequate description of performing an “**autocatalytic reaction**” including, for example, a DNA amplification reaction. I have reviewed the ’544 and ’927 applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. The specifications of the ’544 and ’927 provisional applications do not include a single reference to a DNA amplification reaction. There is no mention in the ’544 or ’927 provisional applications of (for example) **any** DNA amplification reactions, let alone the details necessary to carry out said reactions. Nor has Bio-Rad identified any disclosure in the ’544 or ’927 specifications that discloses DNA amplification reactions.

1057. The applications also lacks adequate description of performing an “**autocatalytic reaction**” in plugs outside of a substrate, including, for example, a DNA amplification in plugs outside of a substrate. I have reviewed the ’544 and ’927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the ’544 and ’927 provisional applications that discloses a DNA

²² I understand that Bio-Rad is not currently claiming that claim 3 is entitled to claim priority to these applications. Plaintiffs’ Corrected Response to Interrogatory No. 1 at 5.

amplification reaction in plugs outside of the substrate.

1058. Bio-Rad has accused. Bio-Rad has taken the following position in its Response to 10X's Interrogatory No. 3:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be "up to several millimeters" in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as "PDMS"), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X's Interrogatory No. 3.

1059. But the '544 and '927 provisional applications do not describe a single "embodiment in which droplets are captured in a capillary tube," let alone a DNA amplification reaction in plugs outside of the substrate.

1060. The specifications of the '544 and '927 provisional applications state:

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'544 application at 28:22-26; '927 provisional application at 27:14-23 (emphasis added). Again, this section speaks to collecting "*solution*" not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in "a standard 1.5 ml

centrifuge tube” or the “[c]ollection . . . using micropipettes”²³ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalesce to allow such collection.

1061. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The ’544 and ’927 provisional applications do not include this figure, or any related discussion. The ’544 and ’927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” ’927 provisional application at 12:16-17; ’544 application at 12:19-13:5,²⁴ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene

²³ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

²⁴ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application at 10:31-11:15.²⁵ However, as discussed below, none of the surfactants described would stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets off the substrate.

1062. There is also, for example, no adequate description of **“providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”** I understand the Court has construed “providing conditions suitable for the autocatalytic reaction” to mean “providing a set of physical and chemical conditions that allow the autocatalytic reaction to occur.” Claim Construction Order at 1. Based

²⁵ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

on Plaintiffs' 4(c) disclosures, Bio-Rad contends that 10X "provid[es] conditions suitable," which includes "the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction," by, for example, "plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,304,193 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 65-67; *see also* Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 40. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4,

the biocompatible conditions within the droplet that allow for enzymes to function and the appropriate levels of reagents for the DNA amplification reaction (all Chromium Products)." *See, e.g.*, Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 41. I have reviewed the '193 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the '193 patent that discloses providing the conditions suitable for a DNA amplification reaction to occur, let alone for a DNA amplification reaction to occur outside of the substrate.

1063. The specification describes a single DNA amplification reaction: "Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences." '193 patent at 44:58-61; *see also* '193 patent at 2:65-67. The specification does not set forth "a set of physical and chemical conditions that allow [any DNA amplification] reaction to occur." There is no mention in the '193 patent of (for example) DNA amplification reactions other than PCR,

let alone “a set of physical and chemical conditions that allow [said] reaction to occur.”

2. *Enablement*

1064. As described in further detail above, I have reviewed various documents regarding Bio-Rad’s infringement position in this case. Based on these documents, it is my opinion that the claims of the ’193 patent are invalid for lack of enablement.

1065. Claims of the ’193 patent, for example, require “**conducting an autocatalytic reaction in plugs.**” I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” within plugs. Infringement of U.S. Patent No. 8,304,193 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1; *see also* Appendix B to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4,

Appendix B to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 8. But the specification of the ’193 patent does not enable the full scope of the limitation, at least under Bio-Rad’s actual and/or apparent application of the claims, without undue experimentation. The claims purport to cover *all* DNA amplification reactions in plugs (whether known or unknown at the time of Ismagilov’s alleged invention), but the specification describes a single DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’193 patent at 44:58-61; *see also* ’193 patent at 2:65-67. The specification does not include a working example of a PCR reaction in plugs. And there is no mention in the ’193 patent of (for example) other DNA amplification reactions, let alone the details necessary to

carry out said reactions. The '193 patent fails to disclose, teach, or suggest how to conduct every “DNA amplification reaction,” and particularly, the “DNA amplification reactions” allegedly performed by 10X,²⁶ within plugs.

I understand that these techniques were developed by 10X years after the priority date of the Ismagilov patents.

1066. As another example, claims of the '193 patent, for example, require an “conducting an autocatalytic reaction in plugs.” I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,304,193 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1, 11, 60, 65, 67, 84-85, 92; *see also* Appendix B to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 2 and 7. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4,

Appendix B to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 8. The claims purport to cover *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov’s alleged inventions), including DNA amplification reactions in plugs *outside of the substrate*. But the specification of the '193 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. The

²⁶ I have not been asked to provide, and have not formed an opinion on whether or not the reactions performed in 10X’s products are “DNA amplification reactions.”

specification does not enable DNA amplification reactions in plugs *outside of the substrate*. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. Surfactants that would enable a POSA to conduct biological reactions within plugs outside of the substrate, let alone DNA amplification reactions in plugs outside of the substrate, are not described in specification of the '193 patent and were not even available as of the alleged priority date of the '193 patent. In fact, surfactants appropriate for this use were not developed or described until 2008—seven years after Ismagilov's alleged invention.

1067. As discussed above, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.”

1068. The surfactants disclosed in the Ismagilov patents would not stabilize droplets under these conditions.

1069. In order to conduct biological assays within microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, preventing coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Holtze at 1632.²⁷ “These requirements [were] particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluortelomer-tails (typically perfluorinated C₆ to C₁₀) . . . do not provide sufficient long-term emulsion stability.” *Id.*²⁸ Even as of 2008, years after the priority date of the ’193 patent, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.*

1070. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. But none of the surfactants disclosed in the specification of the ’193 patent, including the surfactants disclosed in Figure 24, meet the requirements set forth above.

1071. As set forth in the specification, “FIG.24 shows a reaction scheme that depicts

²⁷ Holtze was authored by individuals from Harvard University, Universit’a del Salento, Lecce, Italy, and Raindance Technologies, Inc. Holtze at 1632.

²⁸ When conducting biological assays in droplets, “it is attractive to use a fluorocarbon oil as the continuous phase” and accordingly, a fluorosurfactant to “ensur[e] that drops are stable.” Holtze at 1632.

examples of fluorinated surfactants that form monolayers that are: (a) resistant to protein adsorption; (b) positively charged; and (c) negatively charged. Fig. 24b shows a chemical structure of neutral surfactants charged by interactions with water by protonation of an amine or guanidinium group. FIG 24c shows a chemical structure of neutral surfactants charged by interactions with water deprotonation of a carboxylic acid group.” ’193 patent at 4:63-5:4.

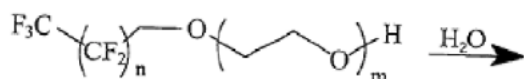


FIG. 24A

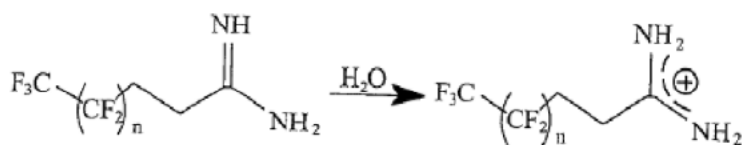


FIG. 24B

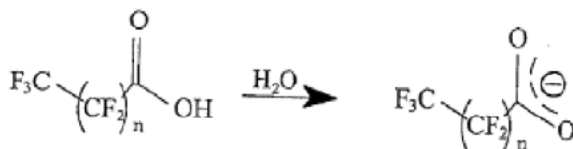


FIG. 24C

Fig. 24

1072. Specifically, Figure 24a “depicts a “fluorinated surfactants containing perfluoroalkyl chains [(red)] and an oligoethylene glycol head group [(blue)].” ’193 patent at 74:26-28.

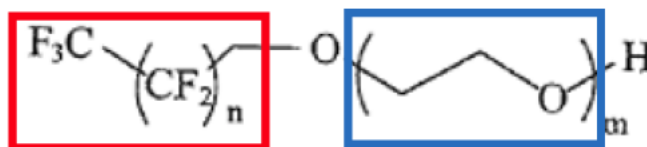


FIG. 24A

1073. The surfactant depicted in Figure 24a is commercially available under the trade

name Zonyl.” See ’193 patent at 20:47-49 (“Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)”); ’193 patent at 76:15-17 (“A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283.”).

1074. Figure 18, depicts the same fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group. ’193 patent at 57:10-12 (“In FIG. 18, plugs are formed in the presence of several solutions of surfactants that possess different functional groups (left side of the diagram)”) (annotation added).

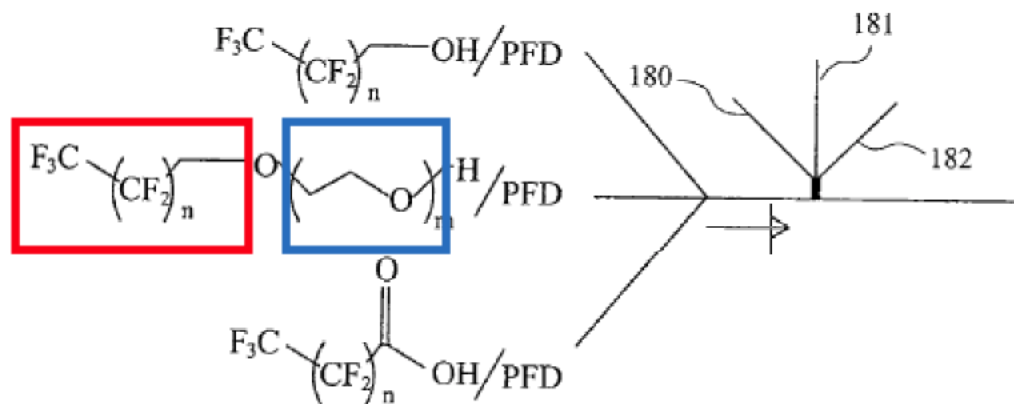


Fig. 18

1075. Unlike the ionic surfactants depicted in Figure 24b and 24c, the surfactant depicted in Figure 24a meets the second requirement set forth above. It will “produce a biologically inert interior surface for the water drops.” As described in the specification of the ’193 patent: “[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.” ’193 patent at 35:63-66. Further, this OEG head group is non-ionic as required for biological assays. Holtze at 1632. But this surfactant does not meet the first requirement set forth above for performing biological assays in

droplets. Specifically, it would not “provide stability to the drops, preventing coalescence.”

1076. The surfactant depicted in Figure 24a contains a “perfluoroalkyl chain[] and an oligoethylene glycol head group.” ’193 patent at 74:27-28. A perfluoroalkyl chain (also referred to as a “perfluoroalkyl tail” of “fluorotelomer-tail”) is not sufficient to stabilize droplets outside of the substrate. As described by Holtze *et al.* “[s]urfactants with short fluorotelomer-tails” like the perfluoroalkyl chain depicted in Figure 24a, “do not provide sufficient long-term emulsion stability.” Holtze at 1632.

1077. I understand that Dr. Jeremy Agresti, Bio-Rad’s R&D Director and a co-author on Holtze *et al.*, confirmed this point. Dr. Agresti was questioned regarding the text copied below from Holtze *et al.*:

However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C₆ to C₁₀) have been used, but do not provide sufficient long-term emulsion stability. Fluorosurfactants with longer fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity.

Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632 (internal citations omitted).

1078. Dr. Agresti confirmed that these statements were accurate at the time they were written in 2008. Agresti Tr. 199:9-13 (“Q. Do you believe that the statements that are made in the article that you just read, those portions that the article that you just read, do you believe those are accurate? A. Yeah, at the time for sure.”).

1079. Further in reference to the following statement in Holtze et al.: “Biological assays thus demand fluorosurfactants with non-ionic head groups; however, there are currently no such surfactants available,” Dr. Agresti confirmed that as of 2008 there were no “fluorosurfactants with nonionic head groups that would stabilize an emulsion long term.” Agresti Tr. 202:2-13 (“Q. And it was true that as of – as of the date of this article, which was 2008, that at least to your knowledge that there were no nonionic fluorosurfactants with nonionic head groups? A. That could stabilize an emulsion long term. We knew that there were fluoro surfactants with nonionic head groups. Q. [W]hat was not known was that there were fluoro surfactants with nonionic head groups that would stabilize an emulsion long term. A. Yes, that’s right.”). Dr. Agresti further confirmed that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, specifically Zonyl, “doesn’t stabilize droplets for PCR.” Agresti Tr. 203:10-19 (Q. Are you familiar with a surfactant known as . . . ZONYL? A. Yes. Q. Has Bio-Rad used that surfactant? A. I can’t say. It’s not in any product. As far as I know it’s never been in any product. Q. Why not? A. As far as I know it doesn’t stabilize droplets for PCR.”).

1080. I understand that named inventor of the ’083 patent Mr. Lewis Spencer Roach, who testified that his “primary contribution” was to developing “fluorinated surfactant[s] [with] hydrophilic head group” Roach Tr. 26:21-23, also confirmed the point that a surfactant with a

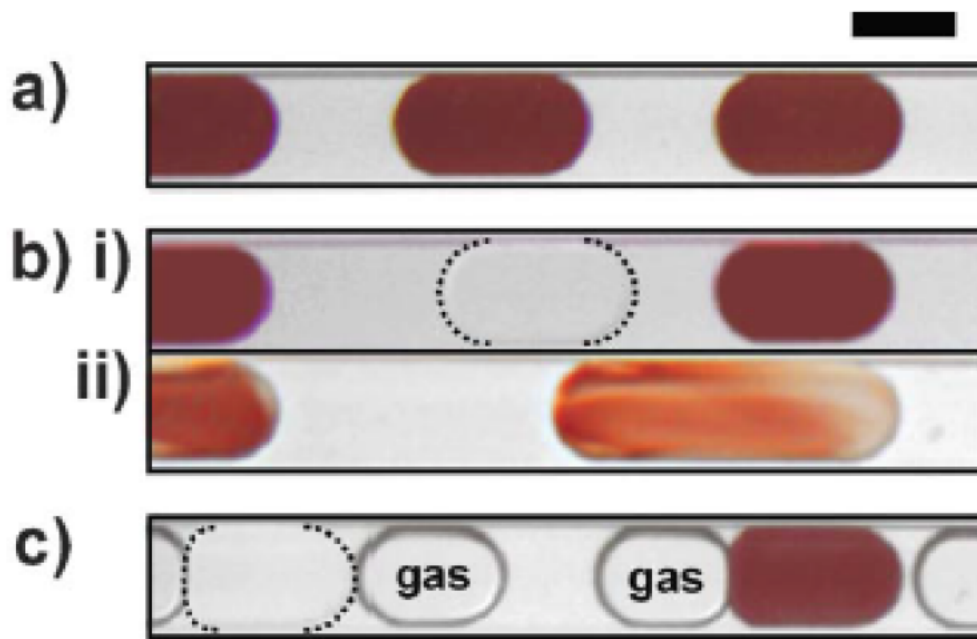
perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl would, not stabilize droplets long term. Mr. Roach testified that “other groups have done a lot of work on preventing coalescence using surfactants” but “I did not personally perform that research.” Roach Tr. 78:15-20. When asked whether “other groups” mean “other people in Dr. Ismagilov’s lab,” Mr. Roach answered that he “believe[d] it was outside of Ismagilov’s group.” Roach Tr. 78:21-79:2. Mr. Roach further testified that the “Rf-OEG surfactant is not optimized for preventing coalescence . . . [t]here are other hydrophilic head groups that are better at controlling adsorption than a simple oligo (ethylene glycol) head group. I think other people have made these.” Roach Tr. at 79:3-12.²⁹ Mr. Roach later confirmed that the “other people” he was referring to were Holtze et al. in 2008. Roach Tr. 80:4-11 (“A. I believe [Exhibit 129 (Holtze et al.)] is what I was just referring to, that other groups had optimized surfactants to – give me just a second. I want to read the conclusions in this paper here. Q. Certainly. A. Yes. This is where I was discussing other groups that have optimized surfactants to prevent coalescence or merging of plugs.”).

1081.

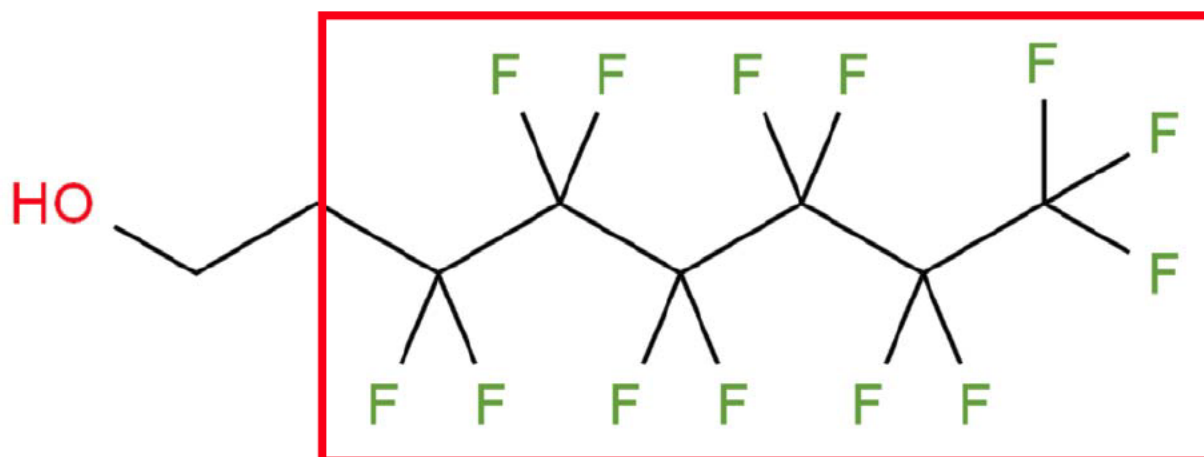
²⁹ The “Rf-OEG” surfactant is a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group. *See* Roach Depo. Ex. 127 (“Perfluorinated-tail, oligoethylene glycol derivatized molecules (Rf-OEG) were selected as a neutral and hypothetically biocompatible surfactants.”); Roach Tr. 51:22-23 (“A. I synthesized the surfactant described in [Exhibit 127], particularly the Rf-OEG surfactant.”).

1082. I agree with Agresti's, Roach's, and Hindson's statements regarding fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl. Such surfactants would not provide stability to drops and prevent coalescence to allow for DNA amplification reactions in microfluidic droplet outside of the substrate.

1083. Dr. Ismagilov himself recognized the potential for coalescence, even between plugs within the substrate. As explained by Dr. Ismagilov, "[d]uring flow, plugs with different chemical composition may move relative to the carrier fluid at different rates and thus move relative to one another allowing adjacent plugs to coalesce (Fig. 2(b))." Adamson at 1181.



1084. Figure 2b above depicts plug coalescence between “[p]lugs of distinct chemical composition.” Adamson at 1181. The carrier fluid is “FC-3283 10:1 PFO (v/v) throughout.” Adamson at 1181. FC-3283 is a fluorinated oil. PFO or 1H,1H,2H,2H-perfluorooctanol is a fluorinated surfactant. The chemical formulation of PFO is depicted below:

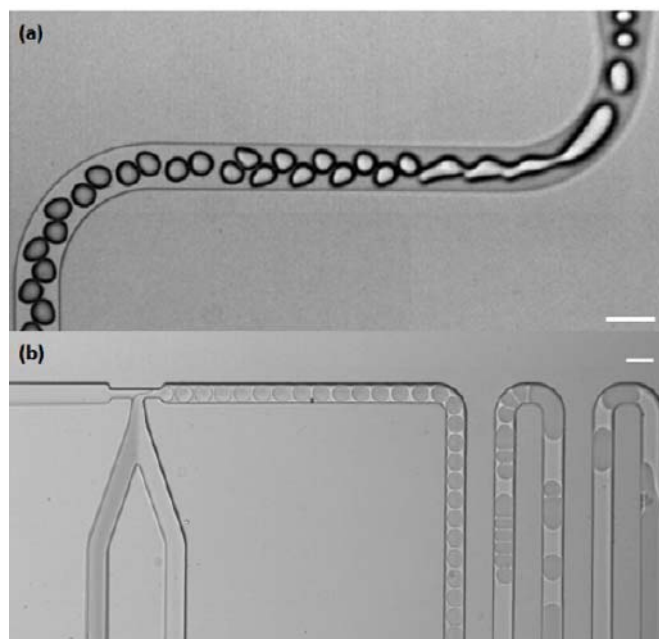


1085. Like Zonyl, PFO contains a short perfluoroalkyl tail (red).

1086. As explained by Dr. Ismagilov, this surfactant was insufficient to prevent

coalescence even within the substrate. *See* Adamson at 1181. Instead “[t]o prevent coalescence, gas bubbles [were] introduced as spacers between plugs to (1) minimize the relative motion of plugs and (2) to act as a physical barrier to prevent the coalescence of adjacent plugs during flow and splitting.” Adamson at 1181.

1087. This potential for droplet coalescence was later described as “[u]ncontrolled.” “Cho Thesis” at Fig. 3.1. This thesis explained: “Perfluorodecalin and 1H,1H,2H,2H-perfluoro-1-octanol combination was used for studying protein crystallization by Ismagilov and his coworkers. The perfluorinated oil and surfactants are advantageous for microdroplet based biochemical applications as they are lipophobic, inert, insoluble in water and compatible with many biochemical molecules. Unfortunately, droplets in perfluorodecalin oil with 1H,1H,2H,2H-perfluoro-1-octanol were not stable and merged with each other under pressure (Figure 3.1(b)).” Cho Thesis at 51. The “[d]roplet generation and uncontrolled coalescence of droplets in perfluorodecalin with 5% v/v 1H, 1H, 2H, 2H-perfluoro-1-octanol” observed is depicted in (b) below:



1088. “Fluorosurfactants with longer fluorocarbon tails” are required for “long-term stabilization” to perform biological assays. Holtze at 1632. As further described by Holtze et al., as of 2008, no such surfactant existed in 2008:

However, the only available PFPE-based surfactants have ionic headgroups, e.g. poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632. Holtze et al. disclose examples of fluorinated surfactants meeting the two requirements set forth above. These surfactants comprise non-ionic polyethylene glycol head groups and perfluorinated polyether tails. Holtze at 1; Figure 2.

1089.

1090. , I understand that RainDance’s droplet products utilize a “biocompatible

surfactant, PEG-PFPE block copolymer.” Plaintiffs’ First and Second Supplemental Response to Interrogatory No. 5. Bio-Rad’s droplet products utilize “Krytox K225 (0.58mM) + perfluorodecanol (0.625 mM) or the BRDG3 triblock fluorosurfactant.” Plaintiffs’ Third and Fourth Supplemental Response to Interrogatory No. 5.

1091. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927, the specifications of the ’544 and ’927 applications do not enable a person of skill in the art to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate without undue experimentation. Surfactants necessary to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate are not described in specification of the ’544 or ’927 provisional applications and were not even available as of the filing date of the ’544 or ’927 provisional applications.

1092. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The ’544 and ’927 provisional applications do not include this figure, or any related discussion. The ’544 and ’927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” ’927 provisional application at 12:16-17; ’544 application at 12:19-13:5,³⁰ and describe the following “[p]referred surfactants”:

³⁰ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

’544 application at 12:19-13:3; ’927 provisional application at 10:31-11:15.³¹ In the context of

³¹ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

conducting biological assays in microfluidic droplets outside of the substrate, each of the surfactants listed—excluding “fluorinated oil” discussed separately in below—would be considered an aqueous soluble surfactant by a POSA, meaning they are introduced in the aqueous phase instead of the oil phase. To conduct biological assays in microfluidic droplets outside of a substrate, a POSA would understand that a continuous phase comprised of a fluorinated oil is preferred, if not necessary. Holtze at 1632. The listed surfactants are non-fluorinated and as such are not soluble in fluorinated oil. Therefore, to use one of the listed surfactants in a system comprising a fluorinated oil, the surfactant needs to be introduced into the aqueous phase. However, when present in the aqueous phase these surfactants would be disruptive to emulsion stability. The hydrophobic portions of these surfactant molecules cause them to populate the aqueous-fluorinated oil droplet boundary, displacing any stabilizing fluorinated surfactant molecules present. This process leads to droplet coalescence rather than stabilization.

1093. “Fluorinated oil” while soluble in fluorinated oil, also would not stabilize droplets to conduct biological assays in microfluidic droplets outside of the substrate. Holtze at 1632 (“[I]t is attractive to use a fluorocarbon oil as the continuous phase However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable.”).

1094. As another example, claims of the ’193 patent require **“providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”** I understand the Court has construed “providing conditions suitable for the autocatalytic reaction” to mean “providing a set of physical and chemical conditions that allow the autocatalytic reaction to occur.” Claim Construction Order at 1. Based

on Plaintiffs' 4(c) disclosures, Bio-Rad contends that 10X "provid[es] conditions suitable," which includes "the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction," by, for example, "plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,304,193 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 65-67; *see also* Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 40. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4,

, the biocompatible conditions within the droplet that allow for enzymes to function and the appropriate levels of reagents for the DNA amplification reaction (all Chromium Products)." Appendix B to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 41. The claims purport to cover providing conditions suitable for *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov's alleged inventions), including conditions suitable for DNA amplification reactions in plugs *outside of the substrate*. But the specification of the '193 patent does not enable the full scope of this limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. The '193 patent fails to disclose, teach, or suggest how to provide all conditions suitable for *any* autocatalytic reaction in plugs outside of the substrate, including all the conditions suitable for a DNA amplification reaction in plugs outside of the substrate.

1095. Bio-Rad has taken the position that "the patents-in-suit disclose a comprehensive toolkit for conducting reactions in chemical droplets." First Supplemental Response to 10X's

Interrogatory No. 3. Specifically, Bio-Rad has taken the position that “the patents-in-suit teach precisely: (1) the types of fluorinated oils and surfactants that have been used throughout the industry for this purpose; (2) the types of microfluidic devices that have been used for this purpose; and (3) the ability to precisely control the composition of droplets so that DNA amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, Bio-Rad provides no explanation for its assertions. As explained above, “the types of fluorinated oils or surfactants” described in the ’193 patent have *not* “been used throughout the industry for this purpose.” Instead, as explained above, each of 10X, RainDance, and Bio-Rad utilize surfactants with non-ionic polyethylene glycol head groups and a perfluorinated polyether tail, or tails. Further, the ’193 specification does not teach “the ability to precisely control the composition of droplets so that DNA amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. The ’193 patent does not describe the “composition of droplets” necessary to conduct any DNA amplification.

3. *Indefiniteness*

1096. It is my opinion that the claims of the ’193 patent are invalid as indefinite because the ’193 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention.

1097. For example, claim 7 of the ’193 patent is indefinite insofar as it lacks an antecedent basis with respect to the claim limitation “**carrier fluid.**” Claim 7 depends on claim 1 which provides:

A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

providing the microfluidic system comprising at least two channels having at least one junction;

flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

flowing an oil through the second channel of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule; and

providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Claim 7 further requires “the carrier fluid further comprises a surfactant.” But there is no reference to “the carrier fluid” in claim 1.

1098. Bio-Rad has taken the position that “one of skill in the art would understand that the term ‘carrier fluid’ is a reference to the ‘oil’ of claim 1.” However, the term “carrier fluid” may be understood to be broader than “the ‘oil’.”

1099. As another example, each claim of the ’193 patent purports to cover “**providing**

conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.” I understand the Court has construed “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified” to mean “providing a set of physical and chemical conditions that allow the reaction to occur.” Claim Construction Order at 1. But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “a set of physical and chemical conditions that allow the reaction to occur.” For example, the specification does not inform, with reasonable certainty which “set of physical and chemical conditions” would allow a DNA amplification reaction to occur. Without an adequate description of what constitutes “conditions suitable” for an autocatalytic reaction, a POSA could not know whether he or she was practicing the claims.

1100. As another example, each claim of the '193 patent purports to cover a plug being “substantially surrounded by an oil flowing through the channel.” But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “**the channel.**” Independent claim 1 of the '193 patent references “a first channel” and a “second channel”:

A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

providing the microfluidic system comprising at least two channels having at least one junction;

flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through *a first channel* of the at least two channels;

flowing an oil through *the second channel* of the at least two channels;

forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the

junction of the at least two channels, the plug being substantially surrounded by an oil flowing through *the channel*, . . .

The claim does not specify whether “the channel” is the “first channel” or the “second channel.”

D. Invalidity Based on Prior Art

1. Obviousness

(a) Invalidity Based on Quake

1101. It is my opinion that Quake discloses and/or renders obvious all elements of claims 1-9, and 11 of the '193 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) Claim 1

1102. The preamble of claim 1 of the '193 patent recites: “**A method for conducting an autocatalytic reaction in plugs in a microfluidic system.**”

1103. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

1104. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

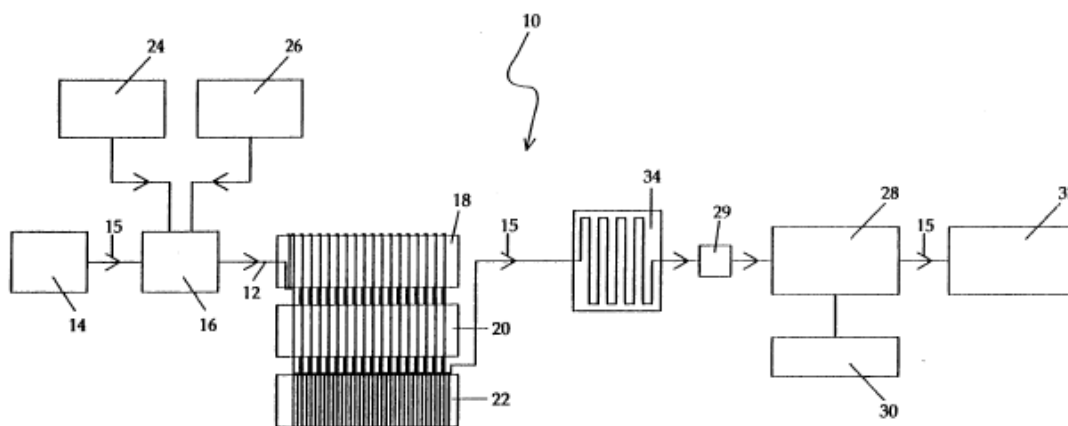
1105. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical*

reactions) or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

1106. Quake also describes a specific type of biochemical reaction. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the ’193 patent explains, PCR is a type of autocatalytic reaction. *See* ’193 patent at 44:58-61 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

1107. While it is my opinion that Quake discloses a method for conducting an autocatalytic reaction in plugs in a microfluidic system, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1108. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1109. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1110. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction that be conducted within droplets in a microfluidic system.

1111. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

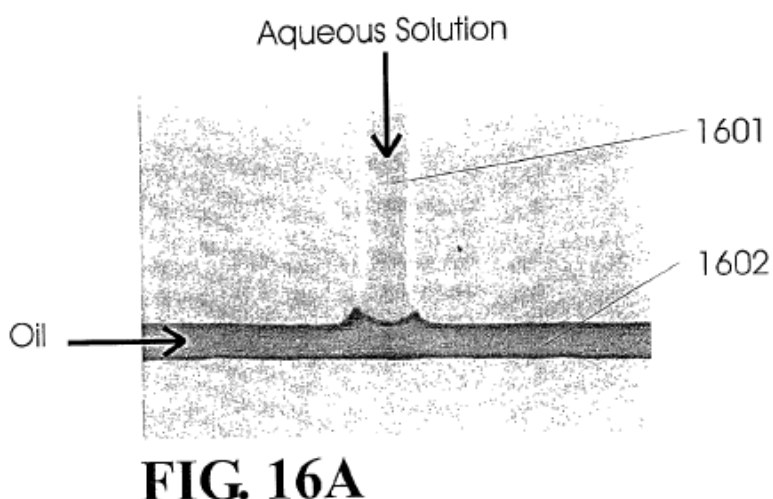
1112. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1113. Quake satisfies this limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

1114. Quake discloses that the microfluidic devices described contain at least two channels having at least one junction. For example, Quake states that “[t]he devices and methods of the invention comprise *a main channel*, through which a pressurized stream of oil is passed, and *at least one sample inlet channel*, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion’ region joins the sample inlet channel to the main channel* such that the aqueous solution can be introduced to the main channel, e.g., at an angle that is perpendicular to the stream of oil.” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the

second fluid are sheared into the main channel.”); Quake at [0068] (“The main channel is typically in fluid communication with an inlet channel or inlet region, which permits the flow of molecules, cells or virions into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a *junction between an inlet region and the main channel of a chip of the invention*”).

1115. Figure 16A in Quake also illustrates this limitation. Figure 16A is reproduced below:



1116. Claim 1 further recites: “**flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.**”

1117. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, *through which a pressurized stream of aqueous solution is passed.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows

through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

1118. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device *and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.* The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample.*” Quake at [0020] (emphasis added). Quake also disclosed that the “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR.*” Quake at [0080] (emphasis added). As the ’193 patent explains, PCR is a type of autocatalytic reaction. See ’193 patent at 44:58-61 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

1119. Quake also made clear that the “flow” of the aqueous fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining *a flowing stream of an aqueous solution* and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce

small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

1120. While it is my opinion that Quake discloses flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1121. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose

(HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1122. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1123. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1124. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and

Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1125. Claim 1 further recites: “**flowing an oil through the second channel of the at least two channels.**”

1126. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

1127. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113]

(emphasis added) (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (*preferably a non-polar fluid such as decane or other oil*) in the main channel.”).

1128. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

1129. Quake describes that the “force and direction” of the flow of carrier fluid oil “can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels).” Quake at [0125].

1130. Quake also made clear that the “flow” of the oil was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and *a flowing stream of an immiscible fluid (e.g., decane)* it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

1131. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.**”

1132. Quake satisfies this limitation. For example, Quake describes the devices and

methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel*, e.g., at an angle that is perpendicular to the stream of oil. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that *the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream thereby forming droplets.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”).

1133. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” Quake at [0020] (emphasis added).

1134. Quake also made clear that his patent application described the forming of droplets by partitioning aqueous fluid with carrier fluid. For example, during prosecution of his patent application, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid

(e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15.

1135. While it is my opinion that Quake discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1136. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification.” Lagally at 567.

1137. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1138. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1139. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1140. Claim 1 further recites: “**the plug being substantially surrounded by an oil flowing through the channel.**”

1141. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

1142. Claim 1 further recites: “**wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.**”

1143. Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

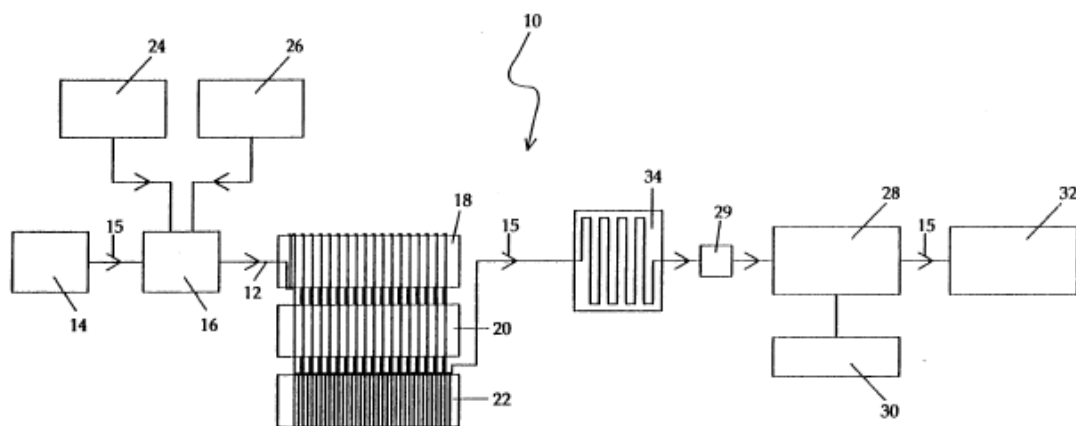
1144. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

1145. Quake also describes a specific type of biochemical reaction. For example, Quake

discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the ’193 patent explains, PCR is a type of autocatalytic reaction. *See* ’193 patent at 44:58-61 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

1146. While it is my opinion that Quake discloses a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1147. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1148. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1149. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1150. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

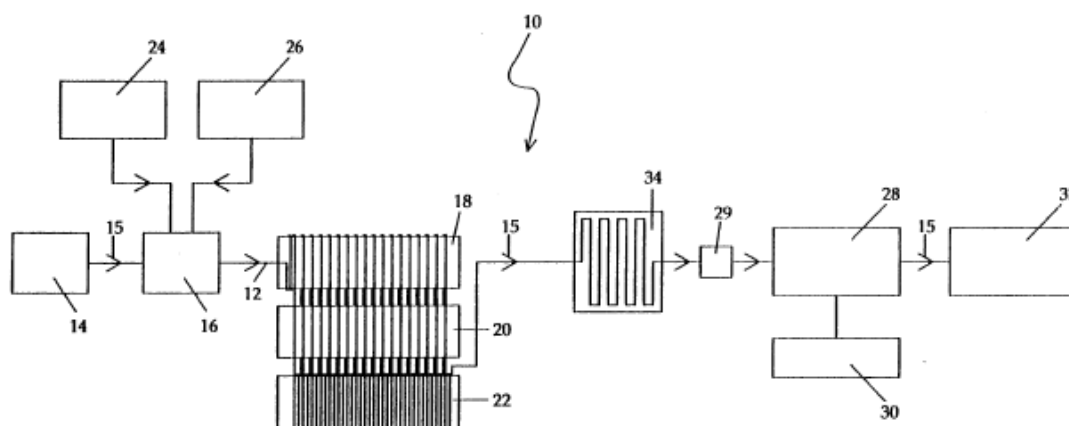
1151. Claim 1 further recites: **“and providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”**

1152. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

1153. While it is my opinion that Quake discloses providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1154. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a

microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1155. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in

the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1156. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1157. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1158. Claim 2 further recites: “**the at least one substrate molecule is a single biological molecule.**”

1159. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules* (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

1160. Quake also makes clear that “[p]referably, *each droplet of this multi-phase mixture encapsulates a single particle*. The droplets are trapped and their boundaries are defined by channel walls, and therefore they do not diffuse and/or mix. Thus, *individual particles or*

molecules can be separately compartmentalized inside individual droplets.” Quake at [0012] (emphasis added); *see also* Quake at [0015] (“In preferred embodiments, the second fluid includes a biological sample that comprises one or more molecules, cells, virions or particles. In exemplary embodiments for detecting and sorting droplet contents, the droplets of the second fluid each contains, on average’s [sic], no more than one particle. For example, in preferred embodiments where the biological material comprises viral particles, each droplet preferably contains, on average, no more than one viral particle.”).

1161. While it is my opinion that Quake discloses that the at least one substrate molecule is a single biological molecule, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1162. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

1163. Claim 3 of the ’193 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

1164. Claim 3 further recites: “**the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.**”

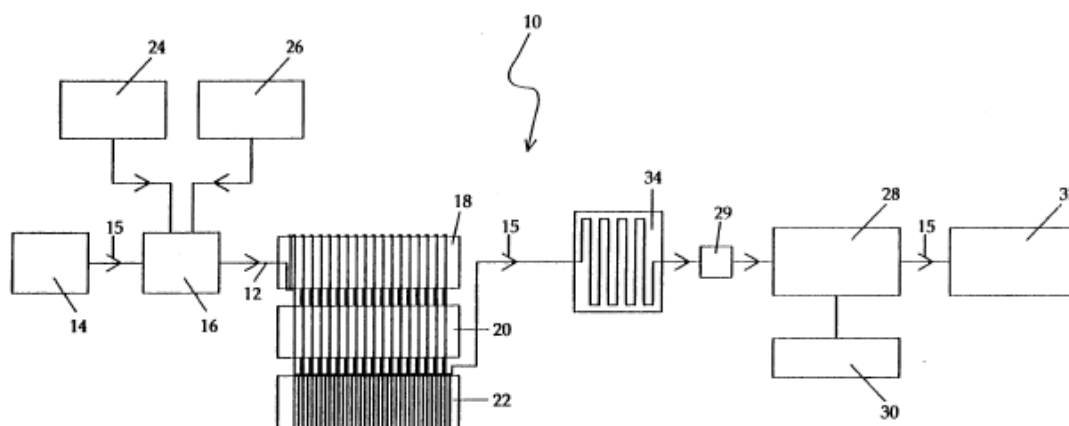
1165. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules (for example, polynucleotides*, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added). Quake defines “polynucleotide” as including “*double and single stranded RNA and DNA*.” Quake at [0052] (emphasis added).

1166. Quake also discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). A person of ordinary skill in the art would have known that PCR requires sample DNA as a reagent. Mullis at Abstract, 2:63-3:1.

1167. While it is my opinion that Quake discloses that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes

along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1168. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally

discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1169. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556.

“[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1170. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1171. Claim 4 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1172. Claim 4 further recites: “**the providing step includes heating.**”

1173. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Quake at [0080]. A person of skill in the art would have known that the PCR reaction required cycles of heating and cooling. Mullis at 9:55-60.

1174. While it is my opinion that Quake discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the

invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1175. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1176. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1177. It also would have been obvious to provide heating to the microfluidic system based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1178. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1179. Claim 5 further recites: “**providing a detector to detect, analyze, characterize,**

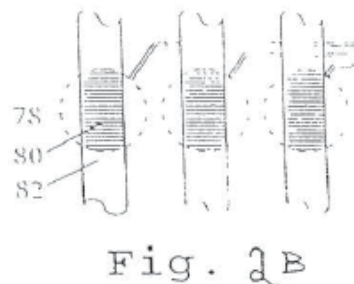
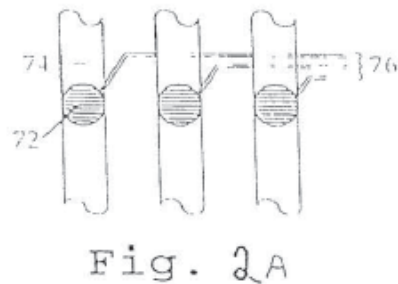
or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

1180. Quake satisfies this limitation. For example, Quake describes that the microfluidic “device of the invention may also comprise a *detection region* which is within or coincident with at least a portion of the main channel at or *downstream of the droplet extrusion region*. The device may also have a *detector, preferably an optical detector such as a microscope*, associated with the detection region.” Quake at [0016] (emphasis added). Quake’s definition of “detection region” is also illustrative:

A “detection region” is a location within the chip, typically within the main channel where molecules, cells or virions to be identified, measured or sorted on the basis of a predetermined characteristic. In a preferred embodiment, molecules, cells or virions are examined one at a time, and the characteristic is detected or measured optically, for example, by testing for the presence or amount of a reporter. For example, *the detection region is in communication with one or more microscopes, diodes, light stimulating devices, (e.g., lasers), photo multiplier tubes, and processors (e.g., computers and software), and combinations thereof, which cooperate to detect a signal representative of a characteristic, marker, or reporter, and to determine and direct the measurement or the sorting action at the discrimination region.*

Quake at [0069] (emphasis added).

1181. Quake also provides diagrams of a detector and detection region. Quake describes that “FIG. 2A shows one embodiment of a detection region used in a sorting device, having an integrated photodiode detector; FIG. 2B shows another embodiment of a detection region, having an integrated photodiode detector, and providing a larger detection volume than the embodiment of FIG. 2A.” Quake at [0025]. Figures 2A and 2B are reproduced below:



Quake at Figs. 2A and 2B.

1182. Indeed, most of the language in Quake describing detectors is almost exactly copied in Ismagilov's 60/379,927 provisional application, to which the patents-in-suit claim priority. See **Exhibit 3** (chart comparing Quake to Ismagilov provisional application).

(vi) *Claim 6*

1183. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1184. Claim 6 further recites: "**the oil is fluorinated oil.**"

1185. Quake satisfies this limitation. For example, Quake describes that "[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water." Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) ("The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*").

1186. I understand that the parties' agreed-to construction for "fluorinated oil" is "an oil that includes one or more fluorine atoms." Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, "may contain additives," including "fluorinated oils." An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an

additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

1187. While it is my opinion that Quake discloses a fluorinated oil, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1188. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1189. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is

required.” *Id.* at 6:46-50.

1190. It also would have been obvious to use a fluorinated oil based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 7*

1191. Claim 7 of the ’193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1192. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1193. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

1194. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at

[0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

1195. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating which minimizes adhesion Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

1196. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(viii) *Claim 8*

1197. Claim 8 of the ’193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1198. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1199. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents

that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

1200. While it is my opinion that Quake discloses a fluorinated surfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1201. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1202. It also would have been obvious to use a fluorinated surfactant based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1203. Claim 11 of the ’193 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

1204. Claim 11 further recites: “**the at least one plug is substantially spherical in shape.**”

1205. Quake satisfies this limitation. For example, Quake explains that “the microfabricated devices of this invention generate *round, monodisperse droplets* (such as those illustrated in Frames J and L of FIG. 19).” Quake at [0093] (emphasis added). Portions of Figure 19 is reproduced below:

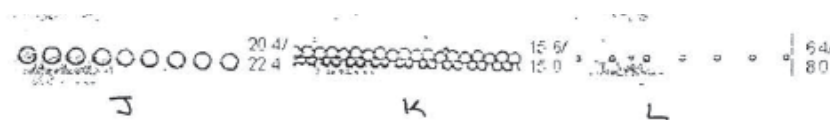
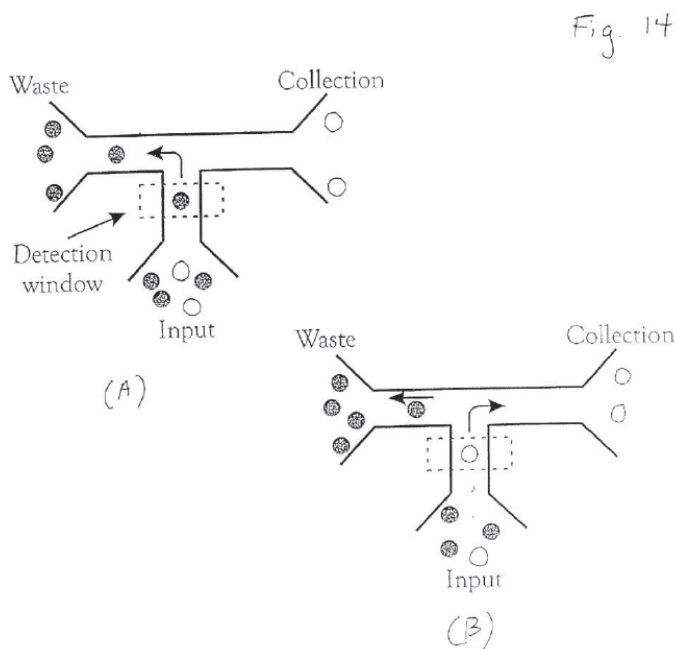


FIG. 19

Quake at Fig. 19. Other figures also demonstrate that in some embodiments, Quake’s droplets are substantially spherical. For example, Figure 14 is reproduced below:



Quake at Fig. 14.

1206. Quake also explains that “[i]n one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels.” Quake at [0092]. Quake also explains that “[f]or particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are *preferably rounded*” Quake at [0091] (emphasis added).

(b) Invalidity Based on Shaw Stewart

1207. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-8, and 11 of the ’193 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1208. The preamble of claim 1 of the ’193 patent recites: “**A method for conducting an autocatalytic reaction in plugs in a microfluidic system.**”

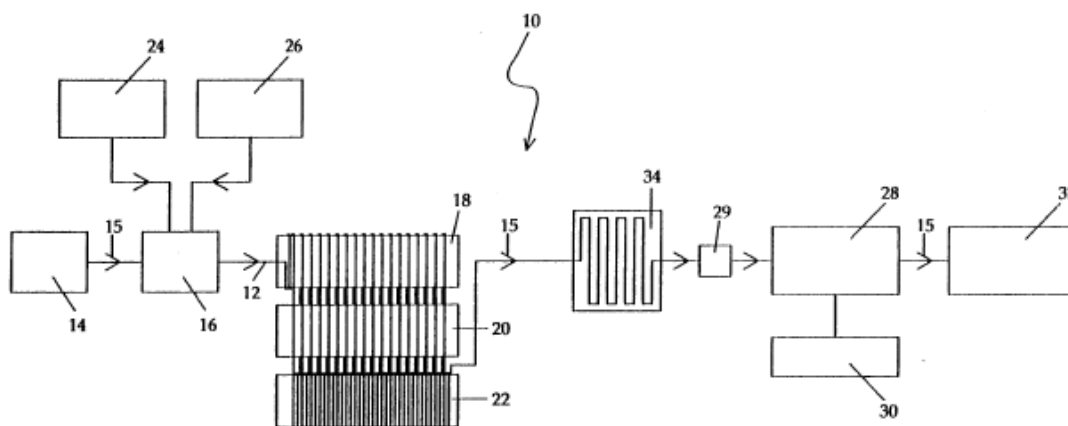
1209. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

1210. Regardless of whether the preamble is limiting, Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

1211. Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86.

1212. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1213. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1214. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1215. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1216. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1217. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1218. Shaw Stewart satisfies this claim limitation. For example, Shaw Stewart described that a microfluidic system with at least two channels comprising at least one junction, as shown in Figure 1, reproduced below:

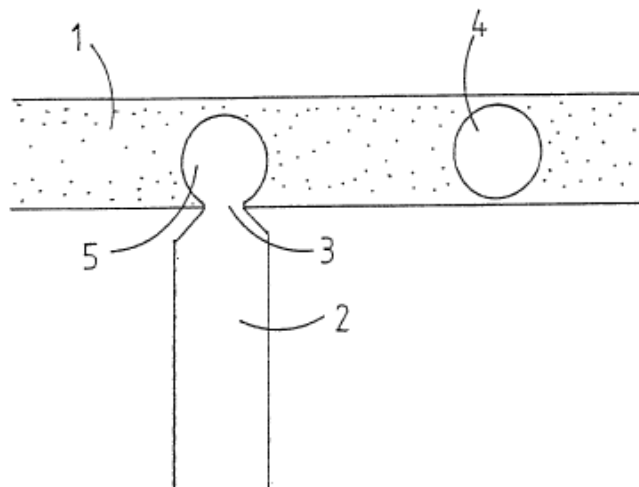


Figure 1.

Shaw Stewart at Fig. 1. In describing the figure, Shaw Stewart stated that “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed

through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1219. Figure 2 of Shaw Stewart, reproduced below, also discloses this limitation.

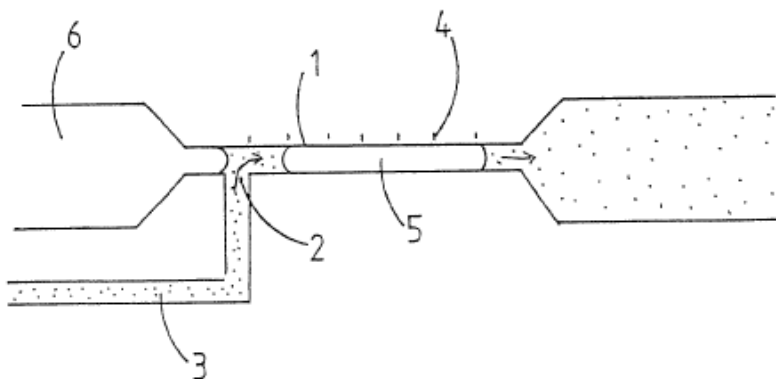


Figure 2.

Shaw Stewart at Fig. 2. In describing Figure 2, Shaw Stewart stated that “[i]n this case the reagent (6) is passed into a tube of considerably narrower bore (1) than the cross-section of the droplets to be produced. The tube is graduated relative to the opening (2) of a side arm (3). The reagent is passed into the tube to a certain graduation (4), whereupon carrier phase is introduced from the side-arm, thus breaking off a droplet to the required size.” Shaw Stewart at 1:96-104.

1220. Claim 1 further recites: “**flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.**”

1221. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a continuous current of carrier phase flows

down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86.

1222. Figure 1 of Shaw Stewart also discloses this limitation.

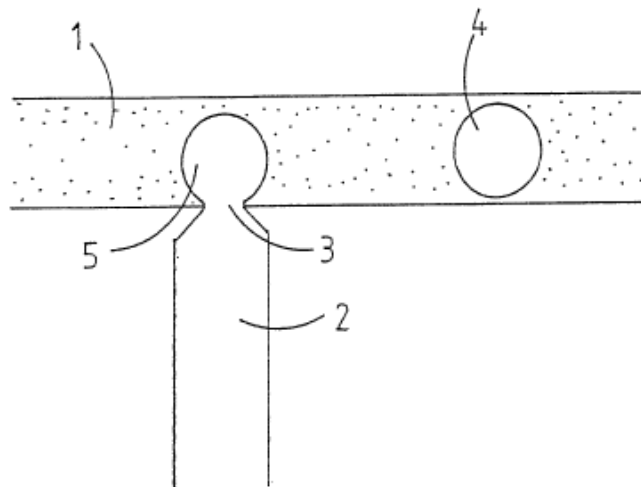


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1223. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

1224. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of

carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1225. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1226. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the

solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1227. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1228. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1229. Claim 1 further recites: “**flowing an oil through the second channel of the at least two channels.**”

1230. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent

liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

1231. Figure 1 of Shaw Stewart also discloses this limitation.

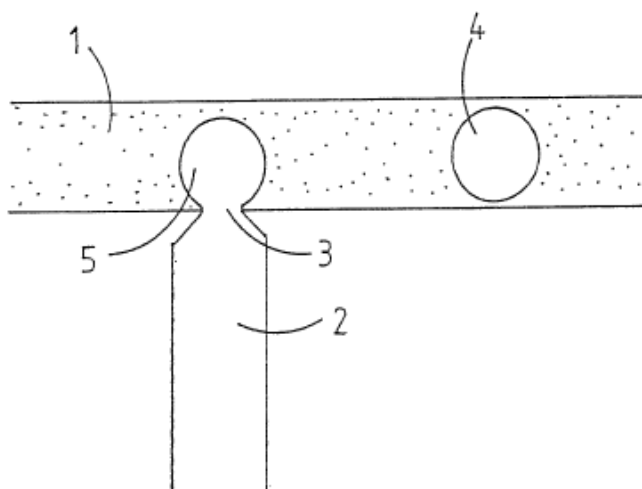


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1232. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the

Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

1233. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.”**

1234. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

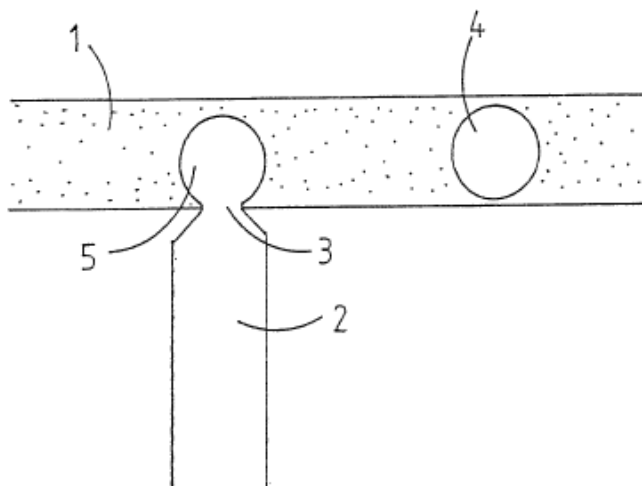


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1235. Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended biological micro-organisms.” Shaw Stewart at 4:30-33.

1236. While it is my opinion that Shaw Stewart discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to

satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1237. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

1238. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and

reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1239. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1240. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1241. Claim 1 further recites: “**the plug being substantially surrounded by an oil flowing through the channel.**”

1242. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

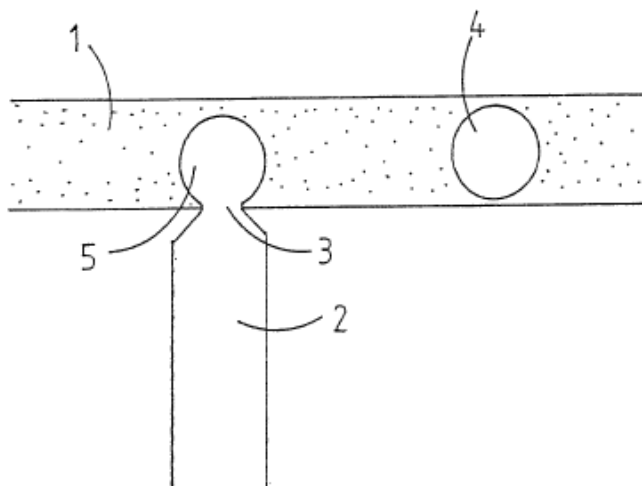


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

1243. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

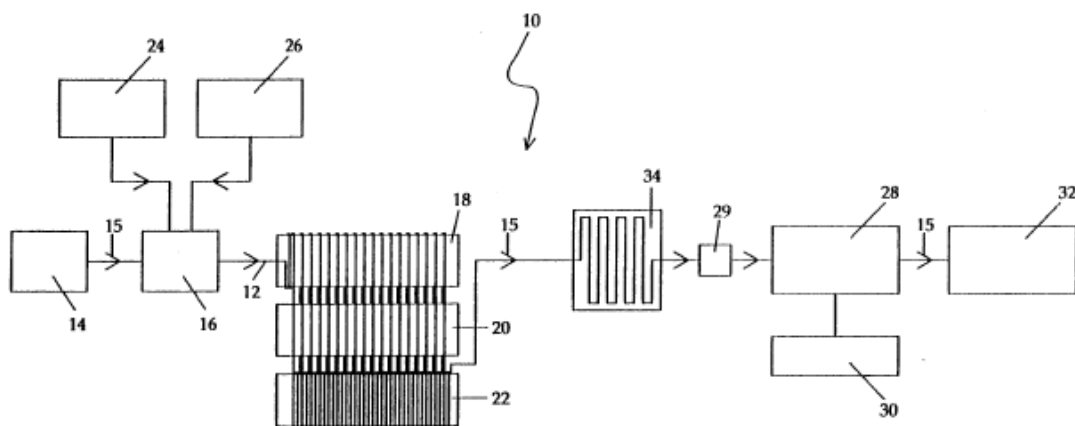
1244. Claim 1 further recites: “**wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.**”

1245. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of

medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86.

1246. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1247. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1248. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate

molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1249. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

1250. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references

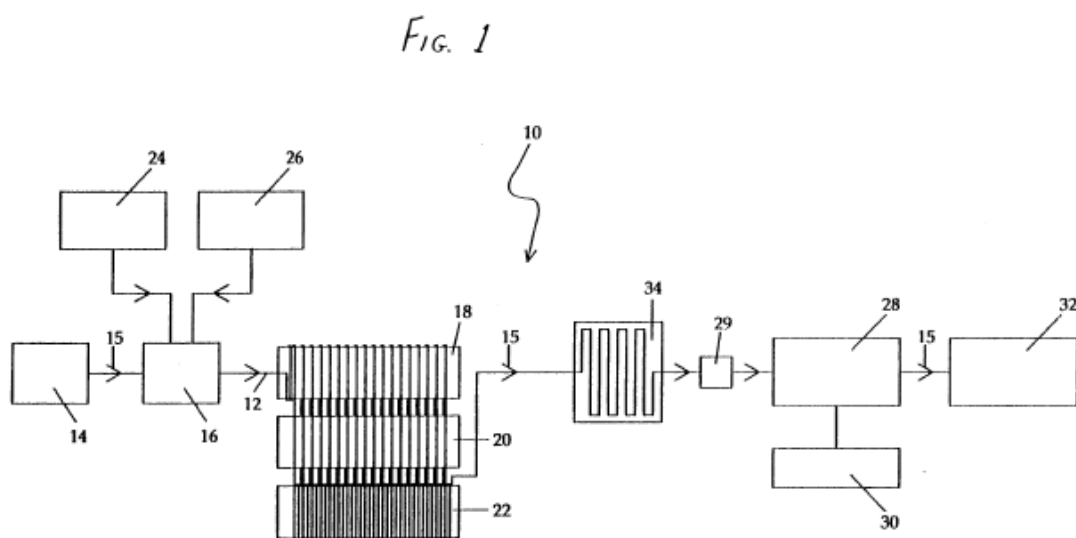
cited therein.

1251. Claim 1 further recites: **“and providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”**

1252. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would allow different types of reactions to take place within droplets. Shaw Stewart described that *“[r]egions of the device can be heated* by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

1253. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.”

Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1254. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-

CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1255. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1256. It also would have been obvious to provide conditions suitable for the

autocatalytic reaction in the at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1257. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1258. Claim 2 further recites: “**the at least one substrate molecule is a single biological molecule.**”

1259. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added). Because Shaw Stewart disclosed that his invention could be applied to, for example, recombinant DNA work, the droplets formed in Shaw Stewart would have been comprised at least one DNA molecule.

1260. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1157-1160, demonstrating how Quake discloses that the at least one substrate molecule is a single biological molecule.

1261. It also would have been obvious to combine the teachings of Shaw Stewart with

one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1262. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

1263. Claim 3 of the ’93 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

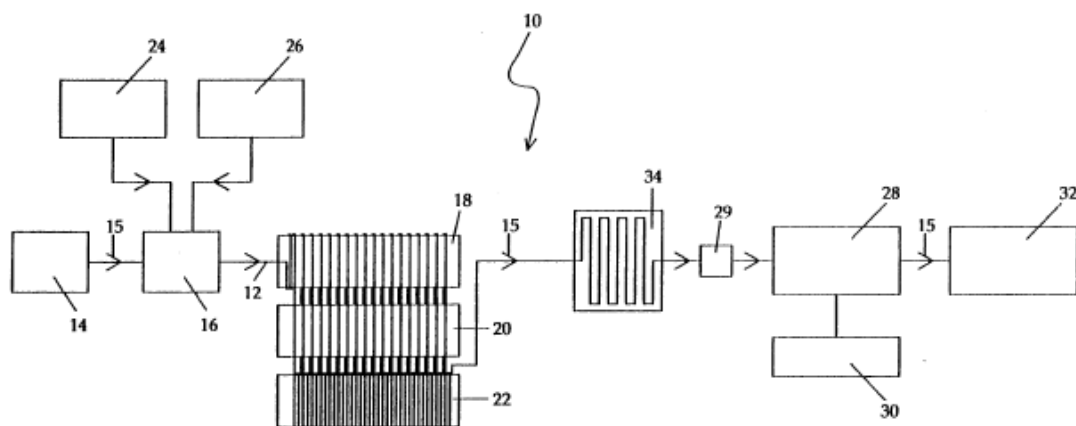
1264. Claim 3 further recites: “**the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.**”

1265. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86

(emphasis added).

1266. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1267. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the

flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1268. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1269. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1270. Claim 4 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1271. Claim 4 further recites: “**the providing step includes heating.**”

1272. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would allow different types of reactions to take place within droplets. Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”).

1273. While it is my opinion that Shaw Stewart discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1274. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal

cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1275. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1276. It also would have been obvious to provide heating to the microfluidic system based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1277. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1278. Claim 5 further recites: “**providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.**”

1279. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Shaw Stewart at 2:59-64; *see also* Shaw Stewart at 2:64-66 (“For example ducts can be formed with two plain transparent walls to form

the sample chambers of spectrophotometers.”). Shaw Stewart also described how reactions resulting in color changes could be detected: “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.” Shaw Stewart at 3:57-60.

1280. While it is my opinion that Shaw Stewart discloses providing a detector, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1187-1182, demonstrating how Quake discloses a detector.

1281. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1282. It also would have been obvious to provide a detector based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 6*

1283. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1284. Claim 6 further recites: “**the oil is fluorinated oil.**”

1285. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated*

hydrocarbons.” Shaw Stewart II at 4 (emphasis added).

1286. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, , it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1287. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1288. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is

required.” *Id.* at 6:46-50.

1289. It also would have been obvious to use a fluorinated oil based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 7*

1290. Claim 7 of the ’193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1291. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1292. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxy, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains **a surfacting agent** which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(viii) *Claim 8*

1293. Claim 8 of the ’193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1294. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1295. Shaw Stewart at least renders obvious this limitation, in light of the background

knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]*urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.* Suitable carrier phases include cholesterol, sodium dioxy, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

1296. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1297. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1298. It also would have been obvious to use a fluorinated surfactant based on Shaw

Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1299. Claim 11 of the '193 patent is dependent on claim '1. I incorporate by reference my analysis with respect to claim 1.

1300. Claim 11 further recites: “**the at least one plug is substantially spherical in shape.**”

1301. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses “[a] method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form *substantially spherical droplets* with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104 (emphasis added); *see also* Shaw Stewart (emphasis added) at 3:128-4:4 (“A method as claimed in claim (1) in which the volume of the chemical reagent is estimated before separation by assuming *said volume is spherical* and measuring the diameter of such a sphere with a microscope or telescope with a graduated eyepiece, after which an adjustment of said volume may be made and said volume separated.”).

(c) Invalidity Based on Burns (2001)

1302. It is my opinion that Burns (2001) discloses and/or renders obvious all elements of claims 1-8, and 11 of the '193 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1303. The preamble of claim 1 of the '193 patent recites: “**A method for conducting an**

autocatalytic reaction in plugs in a microfluidic system.”

1304. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

1305. Regardless of whether the preamble is limiting, Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

1306. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

1307. This reaction is illustrated in Figure 4:

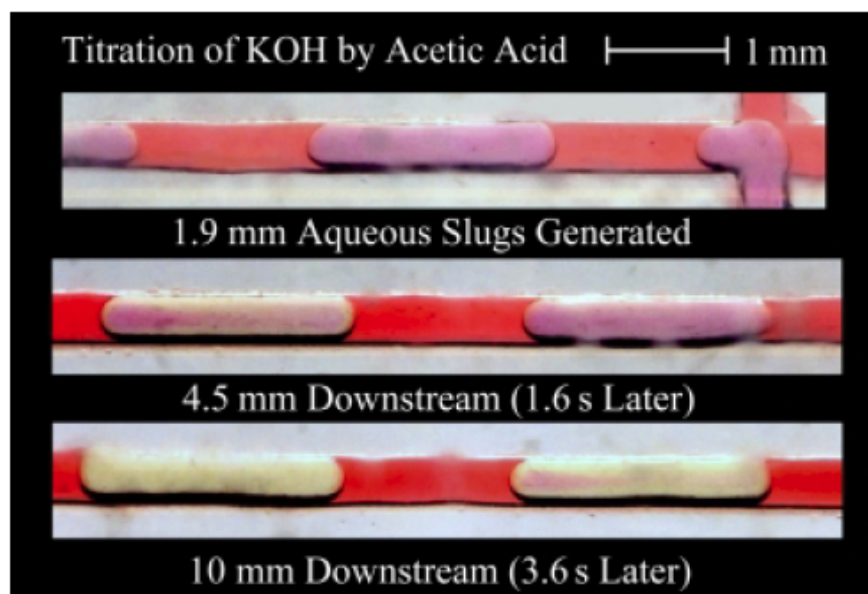
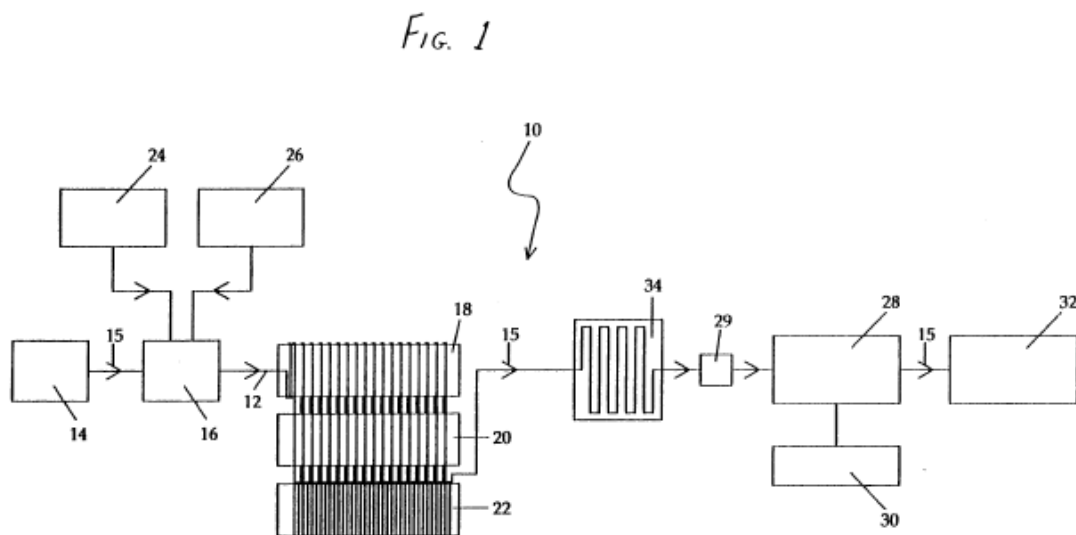


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1308. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46

(referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1309. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying

vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1310. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1311. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may

react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1312. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1313. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1314. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel has been developed. The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a large enhancement in the interfacial mass transfer and the reaction rate. Mass transfer performance data has been obtained for *a glass chip-based reactor in a 380 μm wide channel* by monitoring the extraction of acetic acid from kerosene slugs as they moved along the reactor channel.” Burns (2001) at Abstract (emphasis added).

1315. Figure 4 from Burns (2001) also illustrates a microfluidic system with at least two channels having at least one junction:

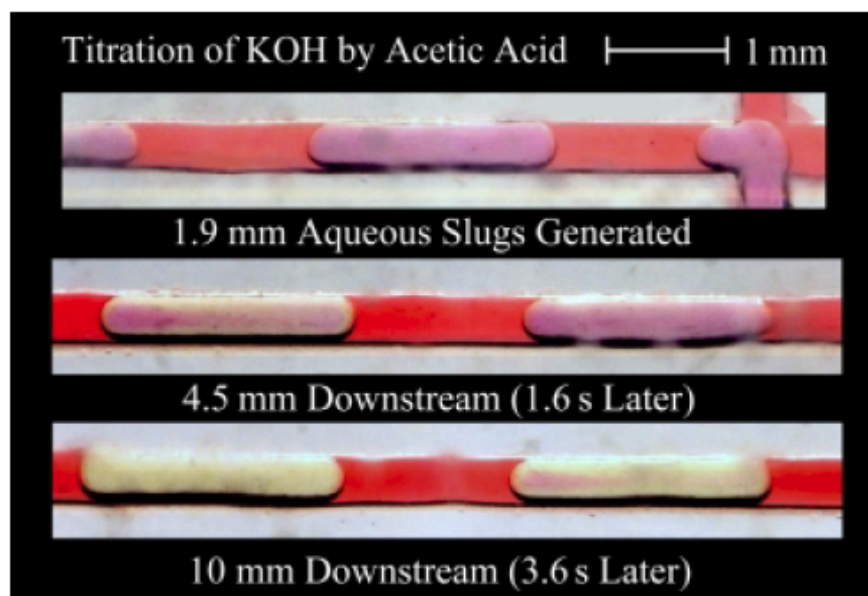


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1316. Claim 1 further recites: “**flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.**”

1317. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

1318. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions

within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1319. This reaction is illustrated in Figure 4:

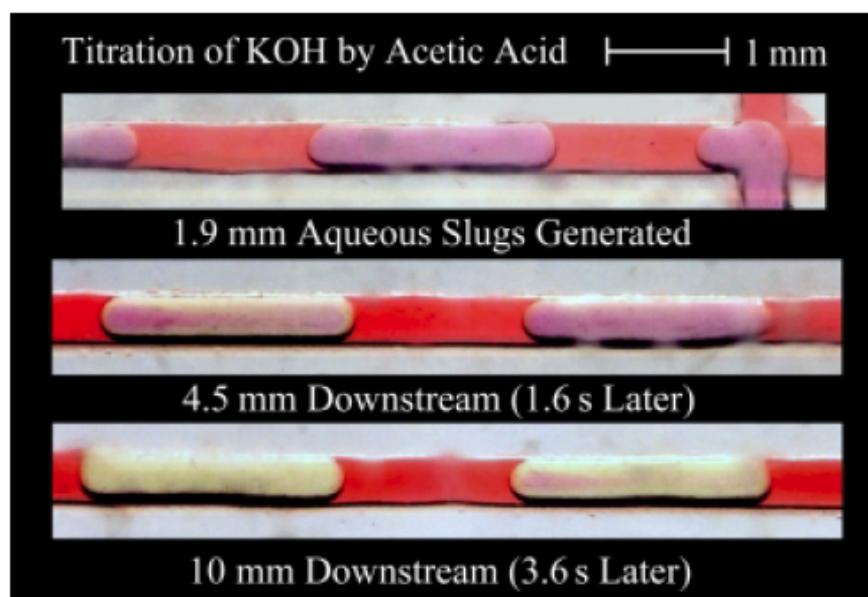


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1320. It also would have been obvious to combine the teachings of Burns (2001) with

one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1321. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1322. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1323. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1324. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1325. Claim 1 further recites: “**flowing an oil through the second channel of the at least two channels.**”

1326. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that

“[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

1327. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1328. This reaction is illustrated in Figure 4:

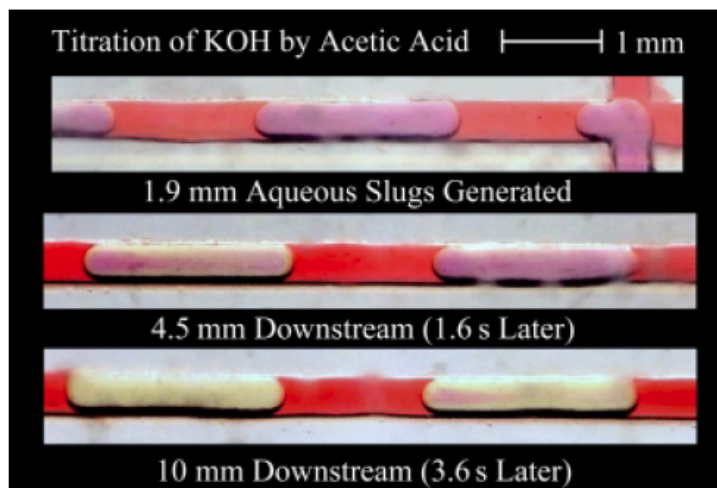


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1329. Claim 1 further recites: “**forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.**”

1330. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

1331. Figure 4 also illustrates this process:

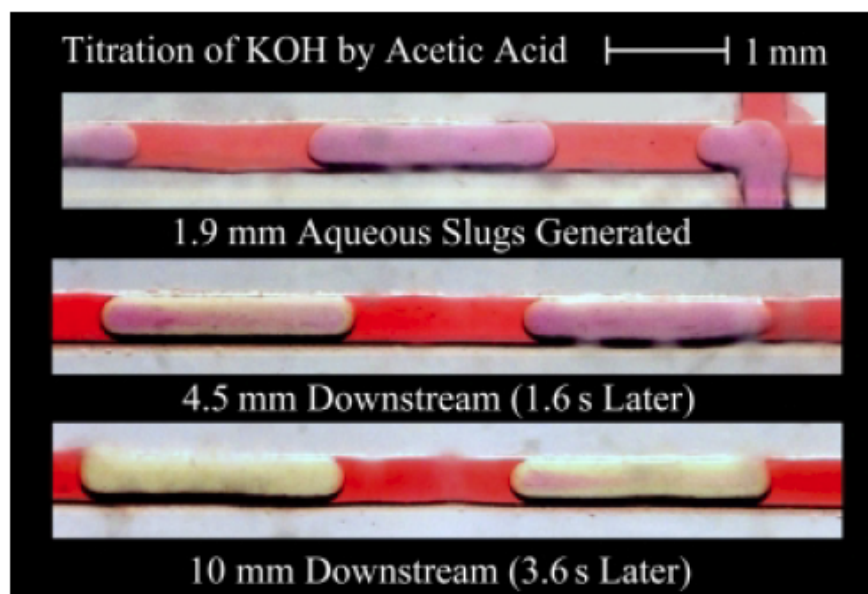


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1332. While it is my opinion that Burns (2001) discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1333. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes

small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

1334. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1335. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).”

Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1336. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1337. Claim 1 further recites: **“the plug being substantially surrounded by an oil flowing through the channel.”**

1338. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

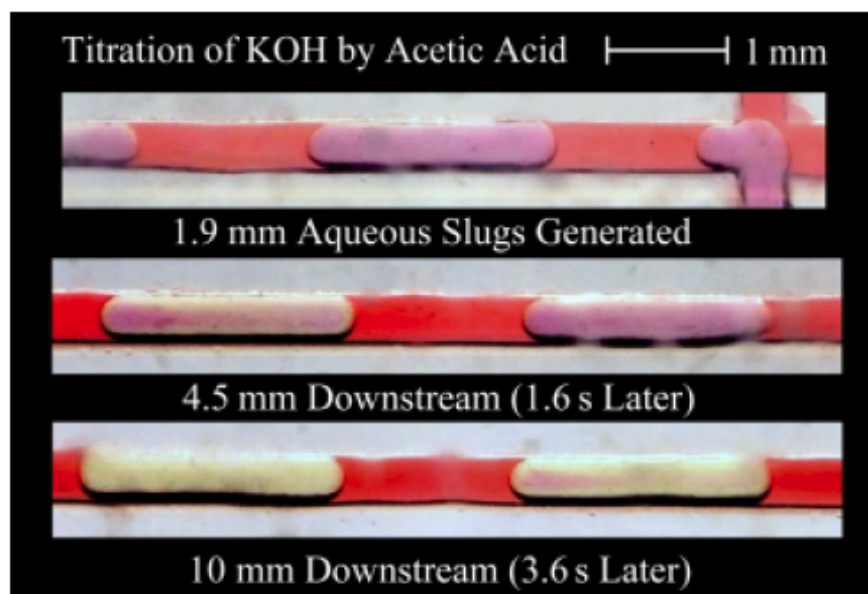


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1339. When a less viscous fluid moves as a “plug” through a more viscous fluid in a micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

1340. Claim 1 further recites: **“wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.”**

1341. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then

diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1342. This reaction is illustrated in Figure 4:

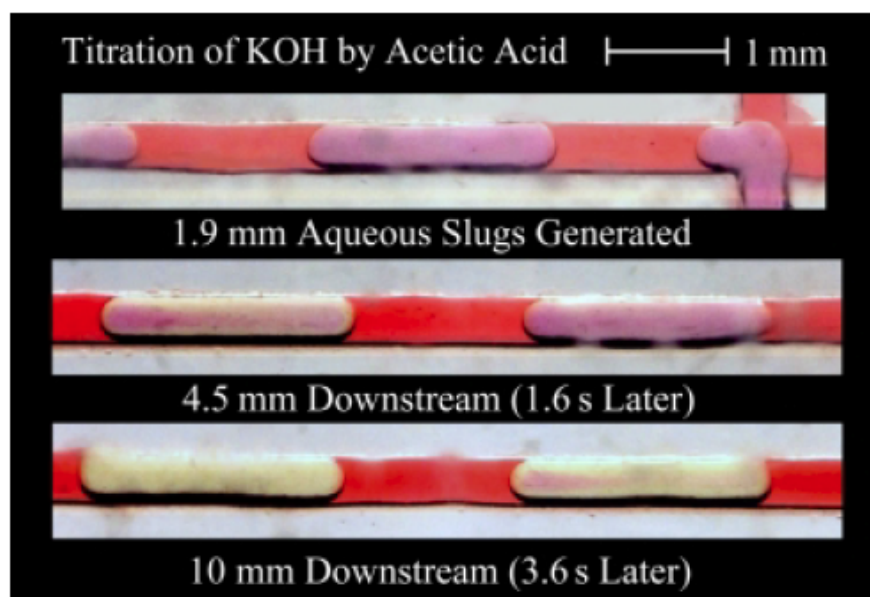


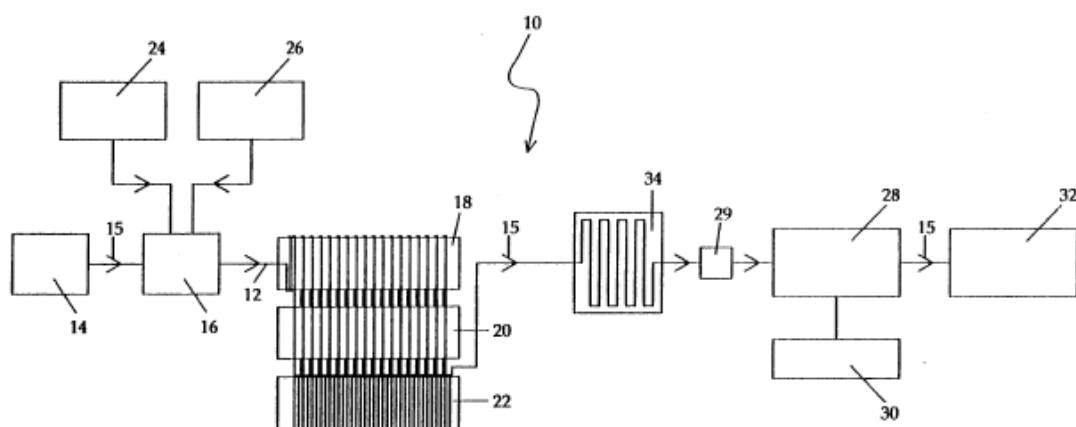
Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1343. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1344. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire

microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1345. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1346. It also would have been obvious to form a plug comprising at least one substrate

molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1347. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1348. Claim 1 further recites: **“and providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”**

1349. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) provides conditions suitable for an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants

within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. ***Kerosene was used as the basis of the organic phase*** with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1350. This reaction is illustrated in Figure 4:

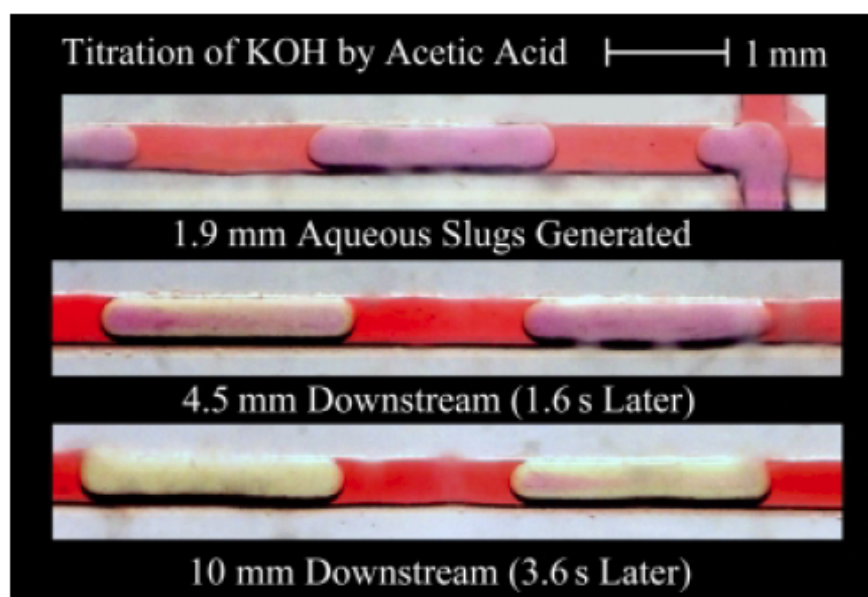


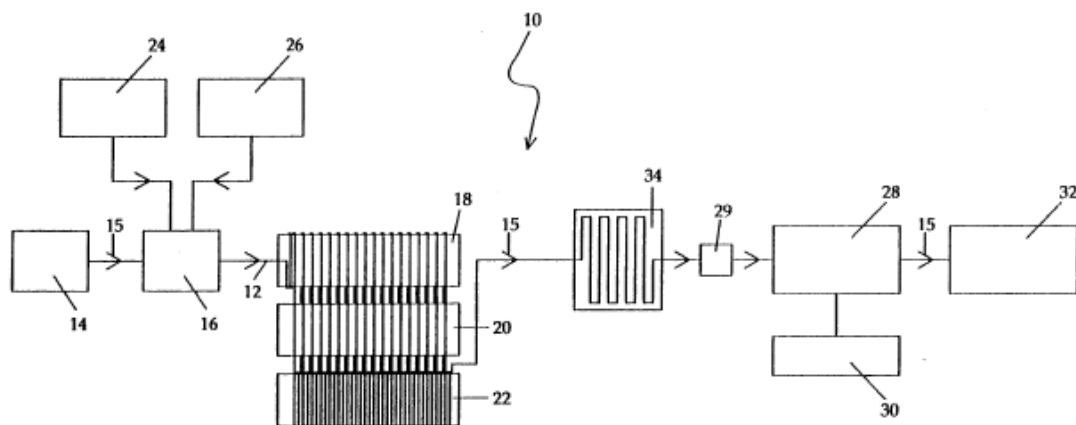
Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1351. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using

the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1352. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1353. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and

reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1354. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1355. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1356. Claim 2 further recites: **“the at least one substrate molecule is a single biological molecule.”**

1357. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1157-1160, demonstrating how Quake discloses that the at least one substrate molecule is a single biological molecule.

1358. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that

“[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1359. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

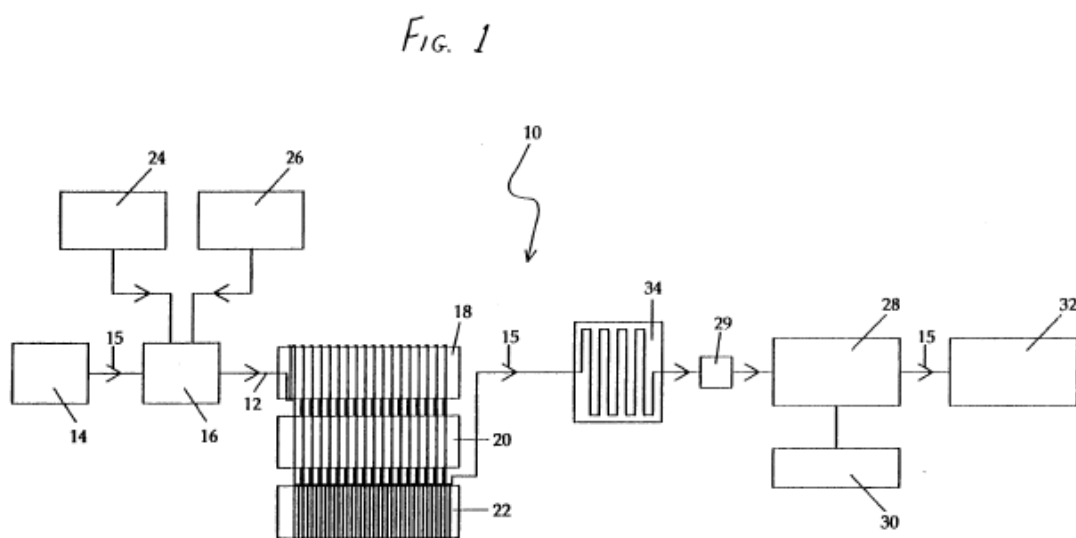
(iii) *Claim 3*

1360. Claim 3 of the '193 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

1361. Claim 3 further recites: **“the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.”**

1362. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal

cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1363. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological

microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1364. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based

on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1365. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1366. Claim 4 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1367. Claim 4 further recites: “**the providing step includes heating.**”

1368. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1369. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL

chamber.” Lagally at Fig. 1.

1370. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1371. It also would have been obvious to provide heating to the microfluidic system based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1372. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1373. Claim 5 further recites: “**providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.**”

1374. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses that “[p]hotographs were taken of the flow using a 35 mm camera with a reversed 24 mm macro lens and up to 55 mm extension tubes. A flash gun was targeted at a white board mounted 6 cm below the chip to provide back illumination. Colour change was observed within the aqueous slug as acetic acid diffused into the aqueous phase reacting with the KOH or NaOH and changing the pH

of the slug.” Burns (2001) at 11.

1375. While it is my opinion that Burns (2001) discloses providing a detector, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1187-1182, demonstrating how Quake discloses a detector.

1376. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1377. It also would have been obvious to provide a detector based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 6*

1378. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1379. Claim 6 further recites: “**the oil is fluorinated oil.**”

1380. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses that “[*k*]erosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation.” Burns (2001) at 11 (emphasis added).

1381. it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1382. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.*d.* 2:20-58.

(vii) *Claim 7*

1383. Claim 7 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1384. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1385. Burns (2001) at least renders obvious this limitation, in light of the background

knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1386. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

(viii) *Claim 8*

1387. Claim 8 of the ’193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1388. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1389. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1390. It also would have been obvious to use a fluorinated surfactant in view of Krafft.

Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1391. It also would have been obvious to use a fluorinated surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1392. Claim 11 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1393. Claim 11 further recites: “**the at least one plug is substantially spherical in shape.**”

1394. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1203-1206, demonstrating how Quake discloses at least one plug that is substantially spherical in shape.

(d) Invalidity Based on Nisisako

1395. It is my opinion that Nisisako discloses and/or renders obvious all elements of claims 1-8, and 11 of the '193 patent, either alone, or in light of the background knowledge of

those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1396. The preamble of claim 1 of the '193 patent recites: “**A method for conducting an autocatalytic reaction in plugs in a microfluidic system.**”

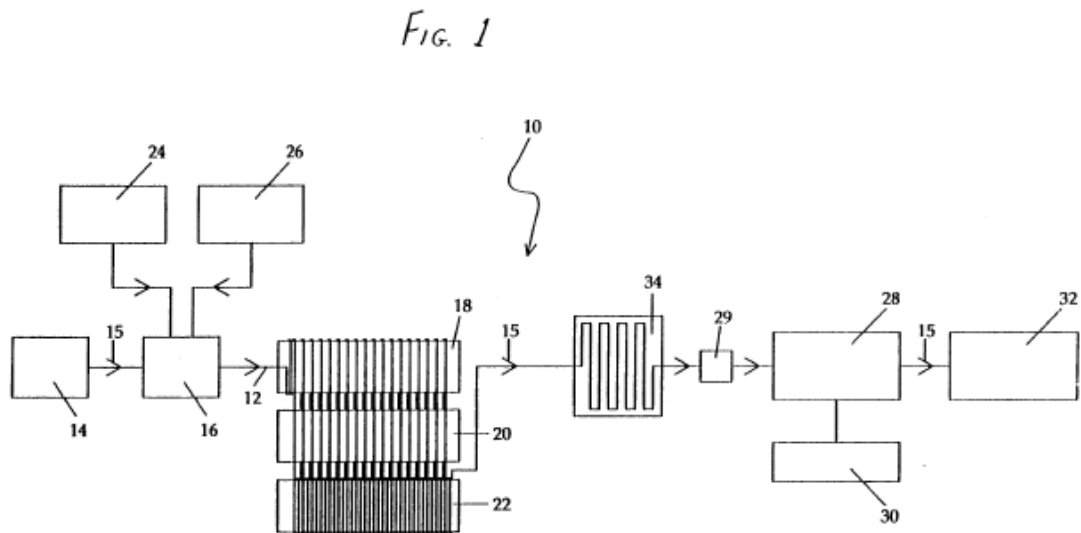
1397. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

1398. Regardless of whether the preamble is limiting, Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network.*” Nisisako at Abstract (emphasis added).

1399. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

1400. It also would have been obvious to combine the teachings of Nisisako with one or

more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1401. It also would have been obvious to conduct an autocatalytic reaction in plugs in a

microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1402. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels

using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1403. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1404. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1405. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1406. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil

as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep.*” Nisisako at Abstract (emphasis added).

1407. The figures in Nisisako also disclose this limitation. For example, Figures 1 and 2 both show a microfluidic system with two channels meeting at a junction:

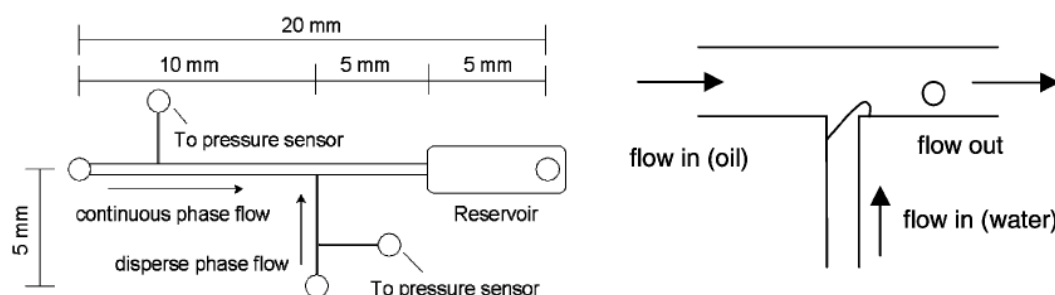


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

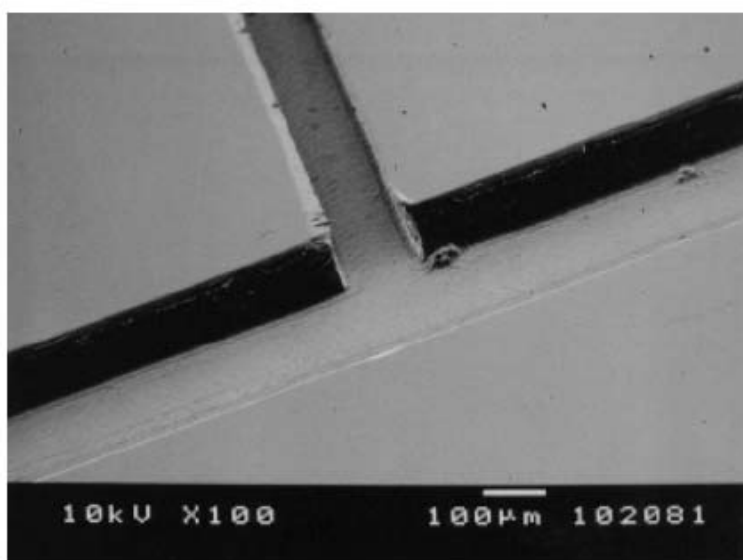


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

1408. Claim 1 further recites: “**flowing an aqueous fluid containing at least one**

substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.”

1409. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps.* No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that *the flow rate is constant.*”).

1410. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

1411. Claim 1 further recites: **“flowing an oil through the second channel of the at least two channels.”**

1412. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil *as the continuous phase* and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water

is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that *the flow rate is constant.*”).

1413. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1414. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose

(HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1415. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1416. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1417. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles

and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1418. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.”**

1419. Nisisako satisfies this limitation. For example, Nisisako describes that *“[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.”* Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating water-in-oil droplets in a microchannel network.”).

1420. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

1421. Nisisako also discloses that “[t]his method of droplet formation is shown schematically in Fig. 1.” Nisisako at 24. Figure 1 is reproduced below:

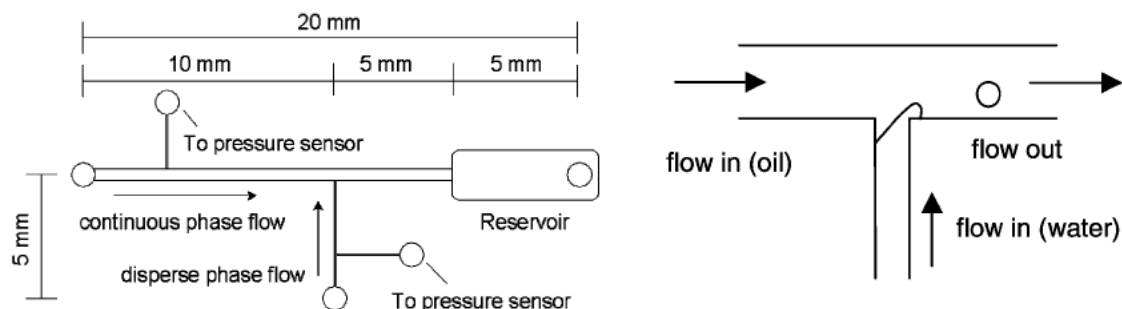


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

Nisisako at Fig. 1. Droplet formation at the T-junction is also illustrated by Figure 3:

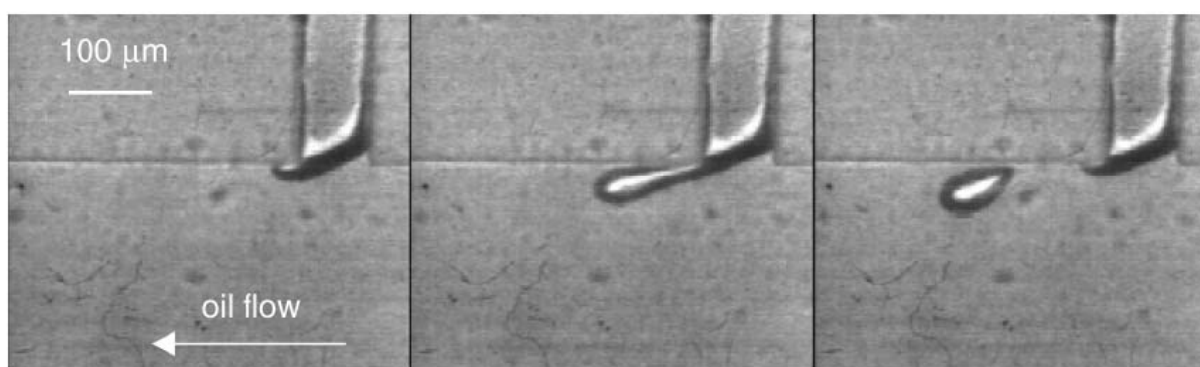


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Fig. 3; *see also* Nisisako at 25 (“Regular-sized droplets of water in oil were generated at the T-junction (Fig. 3).”

1422. While it is my opinion that Nisisako discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes

along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1423. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

1424. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1425. It also would have been obvious to form at least one plug of the aqueous fluid

containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1426. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1427. Claim 1 further recites: “**the plug being substantially surrounded by an oil flowing through the channel.**”

1428. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by the oil:

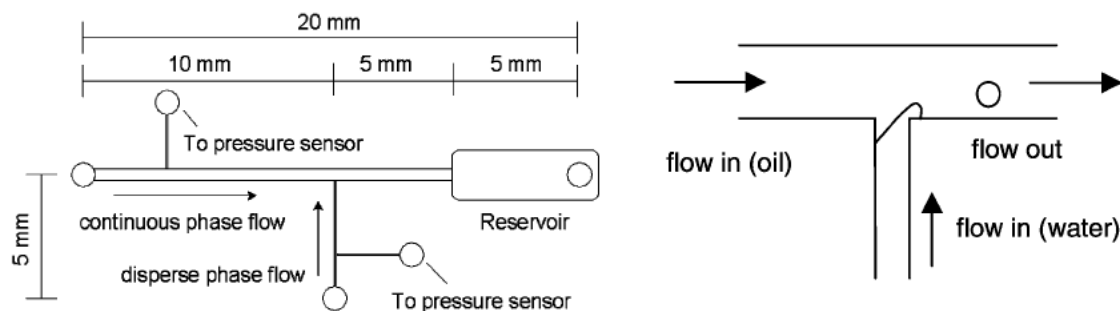


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

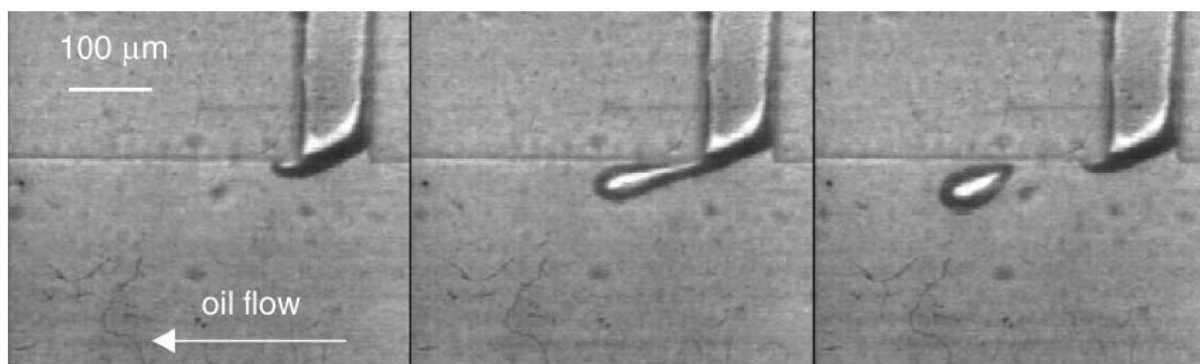


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

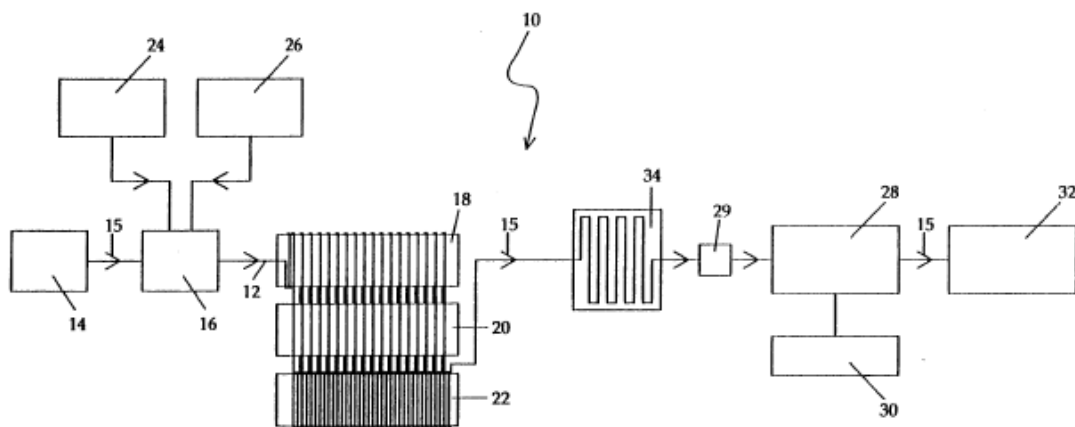
1429. Claim 1 further recites: “**wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.**”

1430. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be

combined with this droplet preparation method in the near future.” Nisisako at 26.

1431. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1432. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1433. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only

local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1434. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

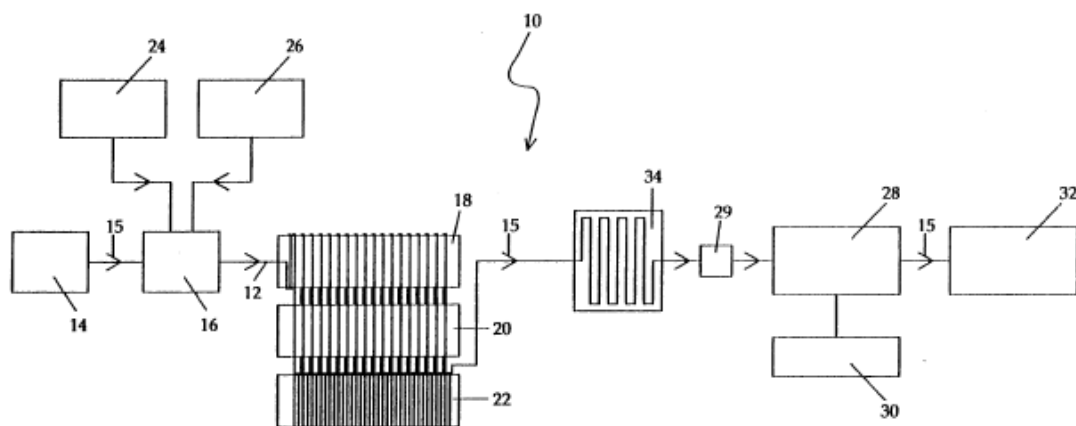
1435. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1436. Claim 1 further recites: “**and providing conditions suitable for the**

autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”

1437. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1438. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1439. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1440. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1441. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1442. Claim 2 further recites: “**the at least one substrate molecule is a single**

biological molecule.”

1443. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

1444. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1157-1160, demonstrating how Quake discloses that the at least one substrate molecule is a single biological molecule.

1445. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1446. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Nisisako in light of the background knowledge of those skilled in

the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

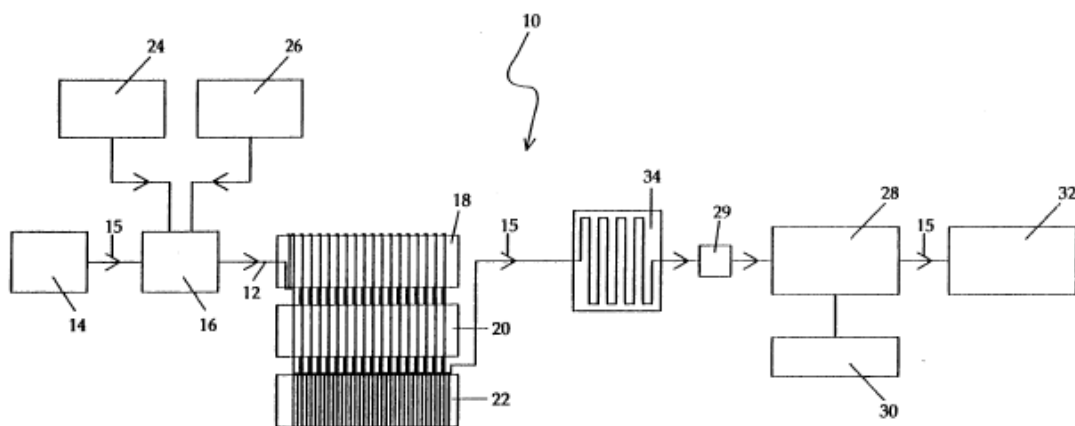
(iii) *Claim 3*

1447. Claim 3 of the '193 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

1448. Claim 3 further recites: **“the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.”**

1449. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1450. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the

flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1451. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1452. It also would have been obvious the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1453. Claim 4 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1454. Claim 4 further recites: “**the providing step includes heating.**”

1455. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1456. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1457. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns

(1996) at 5556.

1458. It also would have been obvious to provide heating based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1459. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1460. Claim 5 further recites: “**providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.**”

1461. Nisisako satisfies this limitation. For example, Nisisako describes that “[d]roplet formation at the T-junction is observed using a microscope (BX50; Olympus, Japan) and a high-speed video camera (FASTCAM-ultima; Photron, Japan).” Nisisako at 25.

1462. While it is my opinion that Nisisako discloses providing a detector, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1187-1182, demonstrating how Quake discloses a detector.

1463. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1464. It also would have been obvious to provide a detector based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 6*

1465. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1466. Claim 6 further recites: “**the oil is fluorinated oil.**”

1467. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1468. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1469. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for

carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1470. It also would have been obvious to use a fluorinated oil based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 7*

1471. Claim 7 of the ’193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1472. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1473. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, , it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.* It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are

provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1474. It also would have been obvious to use a surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 8*

1475. Claim 8 of the ’193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1476. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1477. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1478. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1479. It also would have been obvious to use a fluorinated surfactant based on Nisisako

in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1480. Claim 11 of the '193 patent is dependent on claim '1. I incorporate by reference my analysis with respect to claim 1.

1481. Claim 11 further recites: “**the at least one plug is substantially spherical in shape.**”

1482. Nisisako satisfies this limitation. For example, Nisisako states that “[a] deeper and wider region (depth 1 mm, width 2 mm) is made as a reservoir for observing the droplet size in the *spherical shape*.” Nisisako at 25 (emphasis added). Figure 1 also demonstrates that the droplets form are spherical in shape:

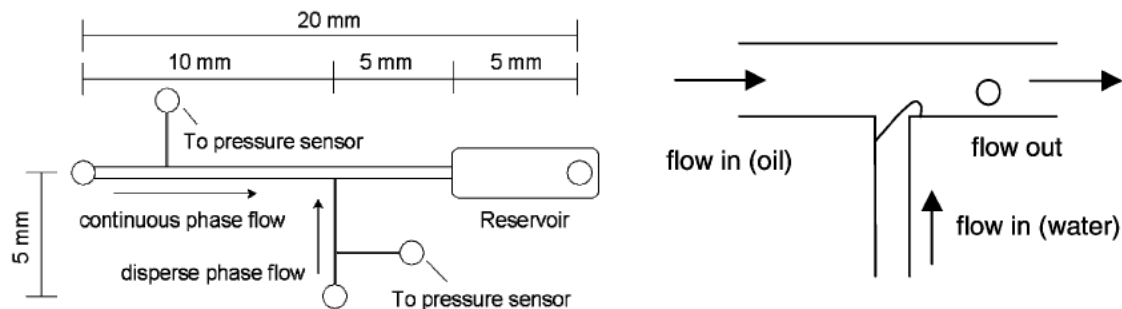


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

Nisisako at Fig. 1.

(e) Invalidity Based on Thorsen

1483. It is my opinion that Thorsen discloses and/or renders obvious all elements of claims 1-8, and 11 of the '193 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1484. The preamble of claim 1 of the '193 patent recites: “**A method for conducting an autocatalytic reaction in plugs in a microfluidic system.**”

1485. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

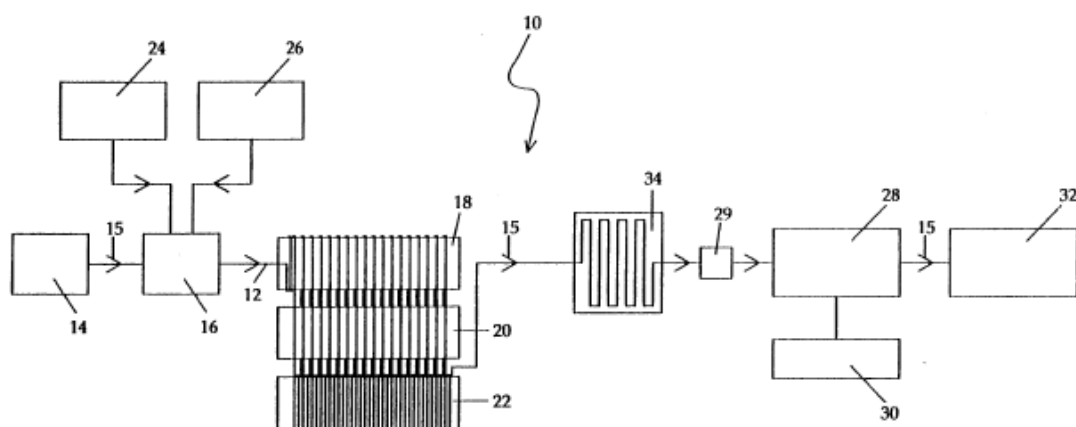
1486. Regardless of whether the preamble is limiting, Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

1487. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

1488. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also

describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1489. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and

to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1490. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1491. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1492. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1493. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1494. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at *the junction of two microfluidic channels* containing water and an oil mixture, respectively.” Thorsen at 4163 (emphasis added); *see also* Thorsen at Abstract (“Here, we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

1495. Thorsen also describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring acrylated urethane (Ebecryl 270, UCB Chemicals) on a silicon wafer

mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately $60\text{ }\mu\text{m}$ wide x $9\text{ }\mu\text{m}$ high, tapering to $35\text{ }\mu\text{m}$ x $6.5\text{ }\mu\text{m}$ in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. Figure 1, showing a microfluidic system with two channels and a junction, is shown below:

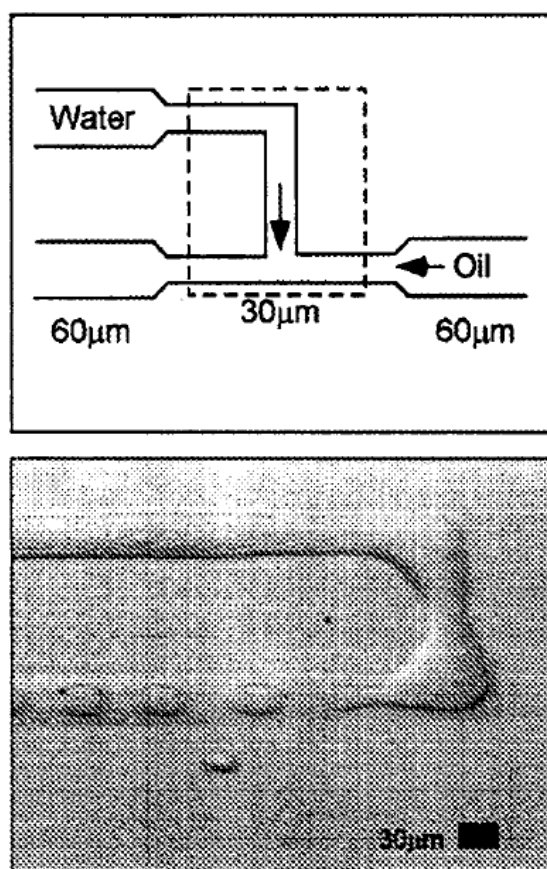


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

1496. Claim 1 further recites: “**flowing an aqueous fluid containing at least one**

substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.”

1497. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, . For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

1498. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

1499. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

1500. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in

a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1501. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1502. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move

discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1503. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1504. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1505. Claim 1 further recites: “**flowing an oil through the second channel of the at least two channels.**”

1506. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture,

respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

1507. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

1508. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.”**

1509. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

1510. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

1511. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant

potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

1512. While it is my opinion that Thorsen discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1513. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification.” Lagally at 567.

1514. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1515. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1516. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1517. Claim 1 further recites: **“the plug being substantially surrounded by an oil flowing through the channel.”**

1518. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

1519. Figure 1, showing droplets surrounded by the oil, is reproduced below:

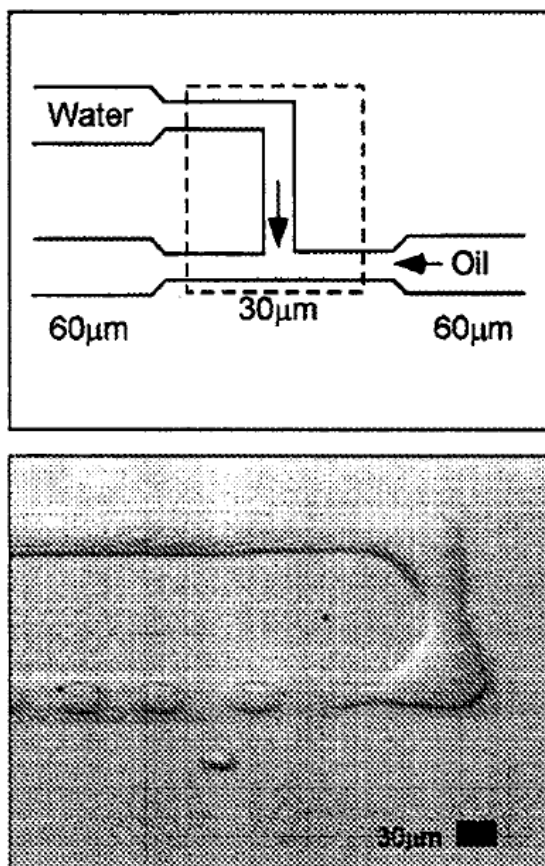


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

1520. Claim 1 further recites: “**wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.**”

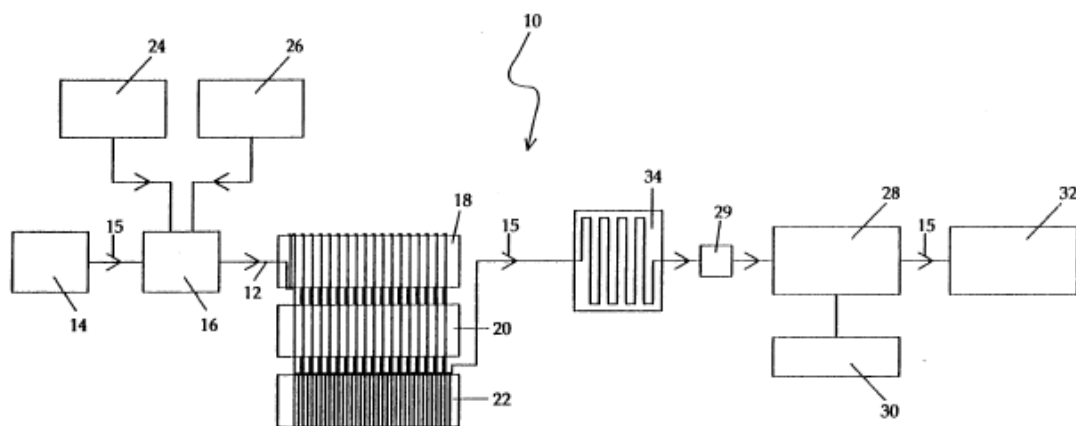
1521. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant

mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

1522. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

1523. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1524. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1525. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1526. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

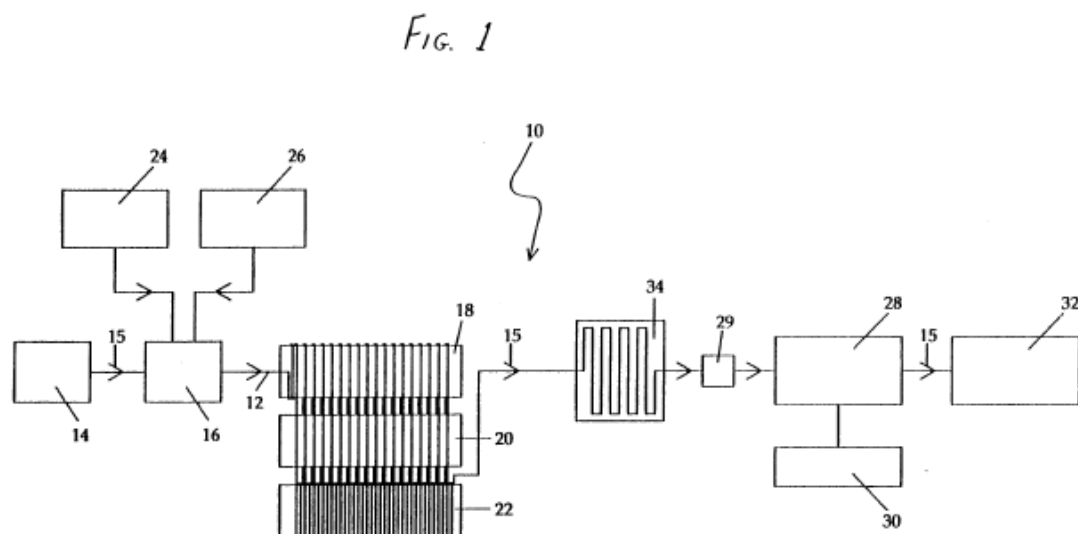
“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1527. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1528. Claim 1 further recites: **“and providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”**

1529. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an

enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1530. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v)

hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1531. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1532. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background

Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1533. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1534. Claim 2 further recites: **“the at least one substrate molecule is a single biological molecule.”**

1535. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to a single biological molecule, such as a DNA or RNA molecule.

1536. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1157-1160, demonstrating how Quake discloses that the at least one substrate molecule is a single biological molecule.

1537. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at

Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1538. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

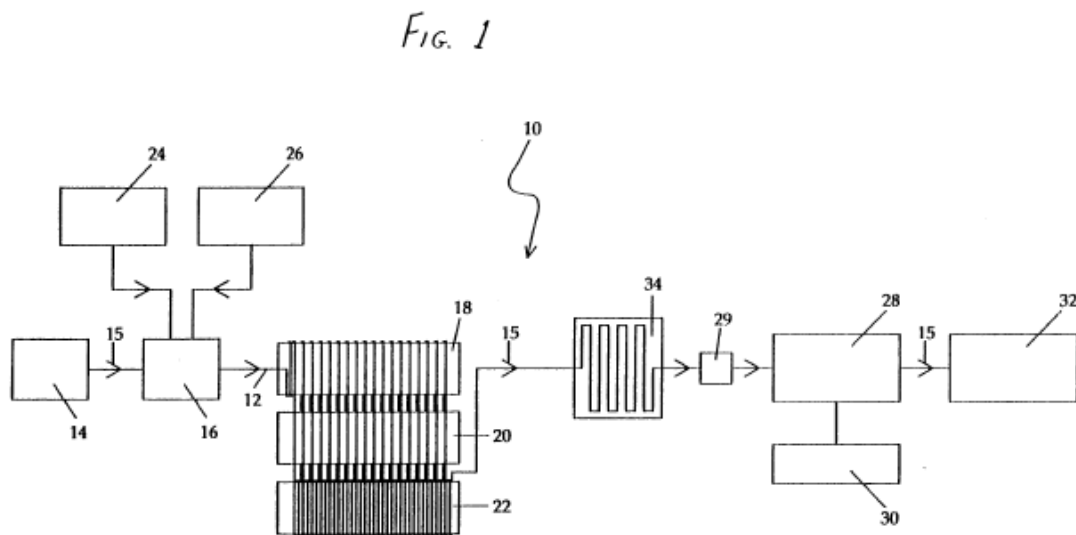
1539. Claim 3 of the '193 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

1540. Claim 3 further recites: “**the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.**”

1541. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to DNA.

1542. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.”

Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1543. It also would have been obvious that the at least one substrate molecule is DNA

and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1544. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns

(1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1545. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1546. Claim 4 of the '93 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1547. Claim 4 further recites: “**the providing step includes heating.**”

1548. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier

fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1549. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1550. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1551. It also would have been obvious to provide heating to the microfluidic system based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1552. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1553. Claim 5 further recites: “**providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.**”

1554. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1187-1182, demonstrating how Quake discloses a detector.

1555. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1556. It also would have been obvious to provide a detector based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 6*

1557. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1558. Claim 6 further recites: “**the oil is fluorinated oil.**”

1559. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are

chemically and biologically stable.” *Id.*

1560. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1561. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1562. It also would have been obvious to use a fluorinated oil based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 7*

1563. Claim 7 of the ’193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1564. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1565. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

1566. Thorsen further describes that “[v]arious oils were tested in the device, including decane, tetradecane, and hexadecane, *combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.*” Thorsen at 4164 (emphasis added).

(viii) *Claim 8*

1567. Claim 8 of the ’193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1568. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1569. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

1570. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1571. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may

also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1572. It also would have been obvious to use a fluorinated surfactant based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1573. Claim 11 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1574. Claim 11 further recites: “**the at least one plug is substantially spherical in shape.**”

1575. Thorsen satisfies this limitation. For example, Thorsen states that “[t]he relative water/oil-surfactant pressures determine the size and spacing between the reverse micelles. The patterns in a rounded channel are more complex, ranging from periodic droplets to ‘ribbons,’ ‘*pearl necklaces*,’ and helical intermediate structures.” Thorsen at 4164 (emphasis added). The figures in Thorsen demonstrate that the droplets in these “pearl necklace” configurations are substantially spherical in shape:

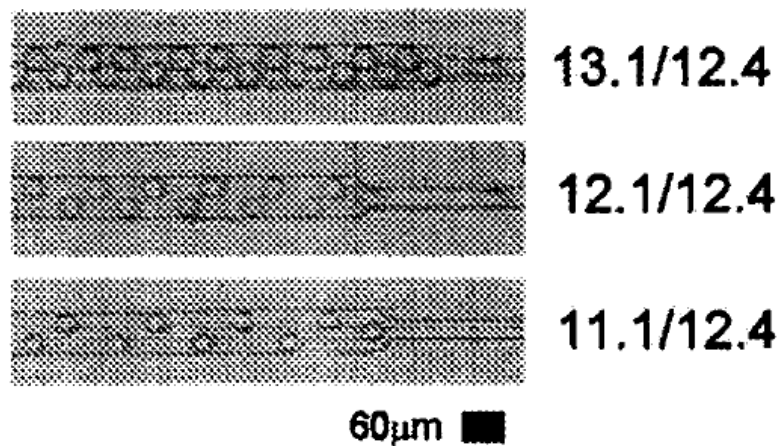


FIG. 2. Reverse micelles in square channels. Photomicrographs show the transition from the 30 μm wide channel to the 60 μm wide channel. Respective pressures for the water and oil/surfactant (hexadecane/2% Span 80) are noted in the figure.

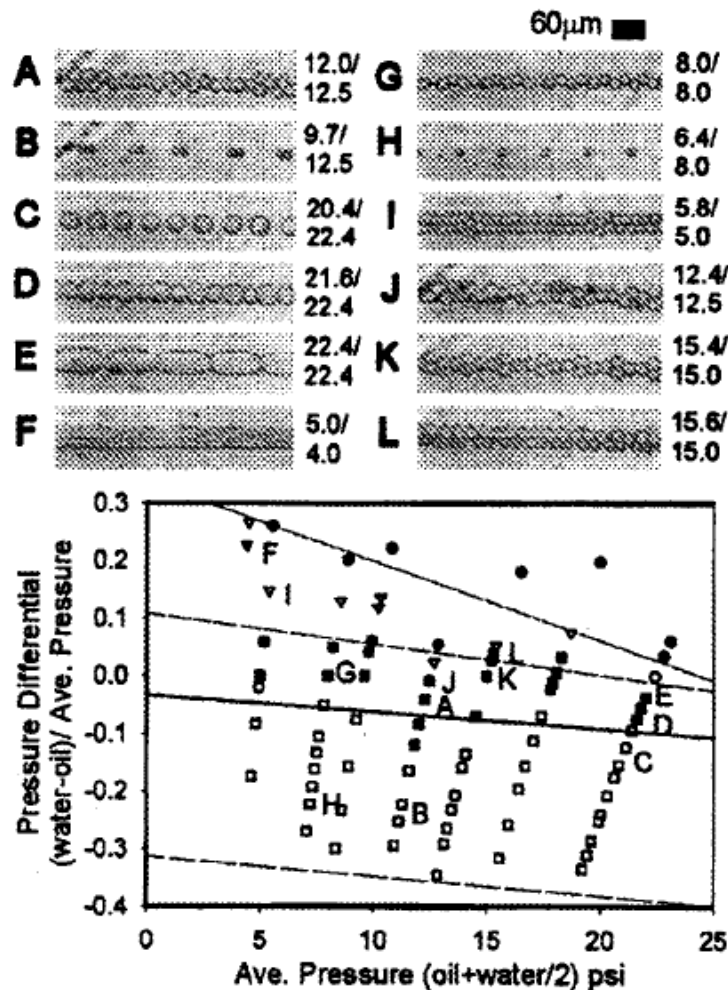


FIG. 3. Droplet patterns in rounded channels at different water and oil/surfactant pressures (noted in the figure) and the corresponding phase diagram depicting the relationship between the oil and water pressure differences and droplet morphology. Solid lines are used to define approximate boundaries between the following droplet states (top to bottom): solid water stream, ribbon layer, pearl necklace, single droplets, and solid oil stream. Symbol definition: solid water stream (solid circle); elongated droplets (open circle); triple droplet layer (solid triangle); double droplet layer (open triangle); jointed droplets (solid square); separated droplet (open square). Photomicrographs show 60 μm channel regions downstream of the point of crossflow.

Thorsen at Figs. 2 and 3.

(f) Invalidity Based on Seki

1576. It is my opinion that Seki discloses and/or renders obvious all elements of claims

1-8, and 11 of the '193 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1577. The preamble of claim 1 of the '193 patent recites: “**A method for conducting an autocatalytic reaction in plugs in a microfluidic system.**”

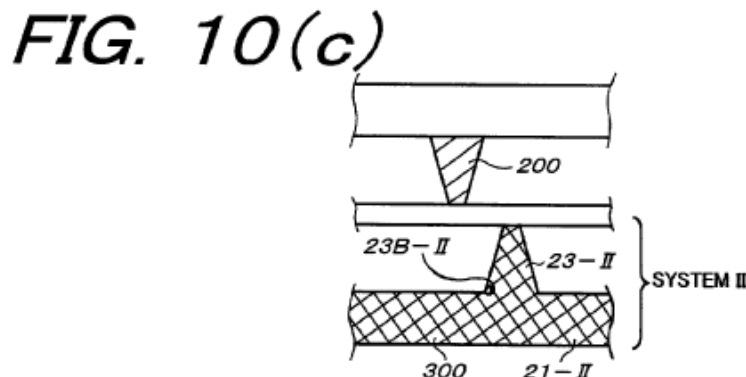
1578. I understand that the Court has construed the preamble of this claim to be limiting with respect to the terms “reaction” and microfluidic systems,” but that the entirety of the preamble is not limiting.

1579. Regardless of whether the preamble is limiting, Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

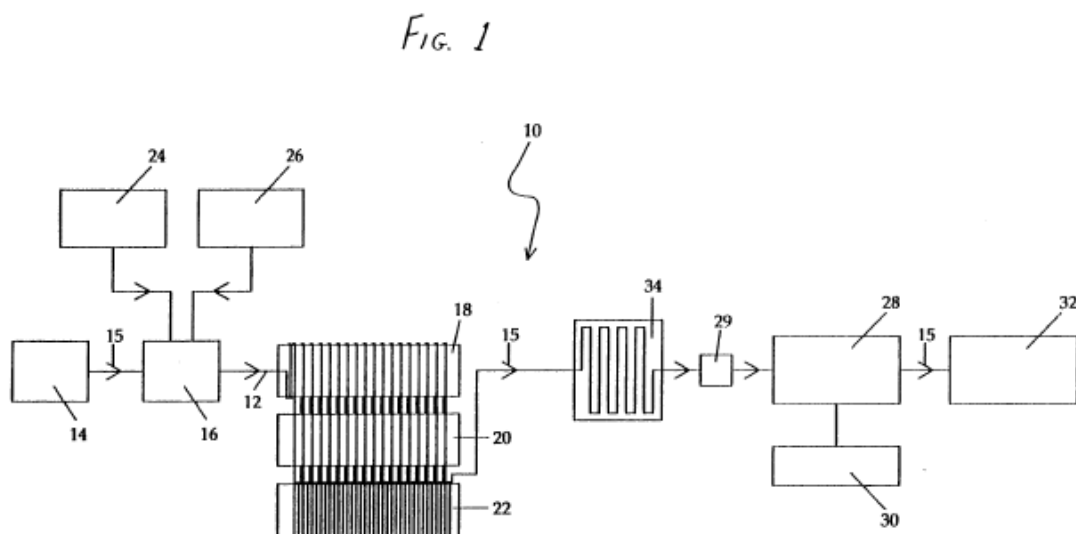
1580. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

1581. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to conduct an autocatalytic reaction in view of Corbett. Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume,

approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1582. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole

orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1583. It also would have been obvious to conduct an autocatalytic reaction in plugs in a microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1584. It also would have been obvious to conduct a reaction in plugs in a microfluidic system in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and

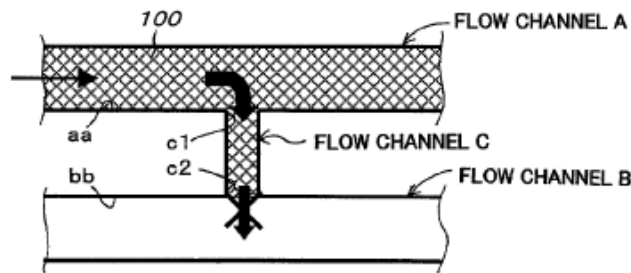
precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

1585. It also would have been obvious to conduct a reaction in plugs in a microfluidic system based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1586. Claim 1 further recites: “**providing the microfluidic system comprising at least two channels having at least one junction.**”

1587. Seki satisfies this limitation. For example, Seki describes a microfluidic system with at least two junctions having at least one junction:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.



Seki at Abstract.

1588. Claim 1 further recites: **“flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels.”**

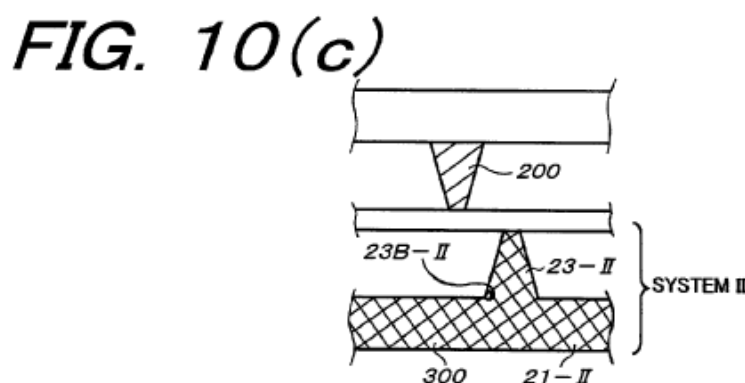
1589. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

1590. Seki describes that a reaction can be conducted within a droplet. “In this case,

coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

1591. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to flow an aqueous fluid with at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Corbett. Corbett describes that “the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid.” Corbett at 4:24-35.

1592. It also would have been obvious to flow an aqueous fluid containing at least one

substrate molecule and reagents for conducting an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1593. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1594. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction

zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1595. It also would have been obvious to flow an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1596. Claim 1 further recites: **“flowing an oil through the second channel of the at least two channels.”**

1597. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

1598. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1126-1130, demonstrating how Quake discloses an immiscible carrier fluid that is an oil.

1599. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use an oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1600. It also would have been obvious to use an oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1601. It also would have been obvious to use an oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be

suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1602. It also would have been obvious to use an oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1603. Claim 1 further recites: **“forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.”**

1604. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

1605. While it is my opinion that Seki discloses forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For

example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62.

1606. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification.” Lagally at 567.

1607. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and

reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1608. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

1609. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1610. Claim 1 further recites: “**the plug being substantially surrounded by an oil flowing through the channel.**”

1611. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow

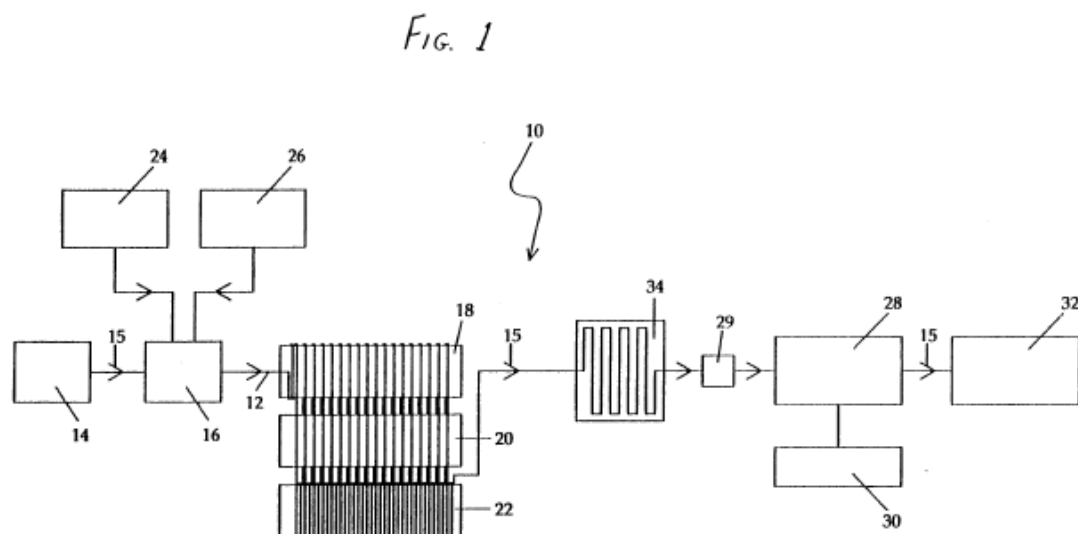
channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

1612. Claim 1 further recites: “**wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule.**”

1613. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

1614. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s)

to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1615. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v)

hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1616. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1617. It also would have been obvious to form a plug comprising at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate

molecule in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

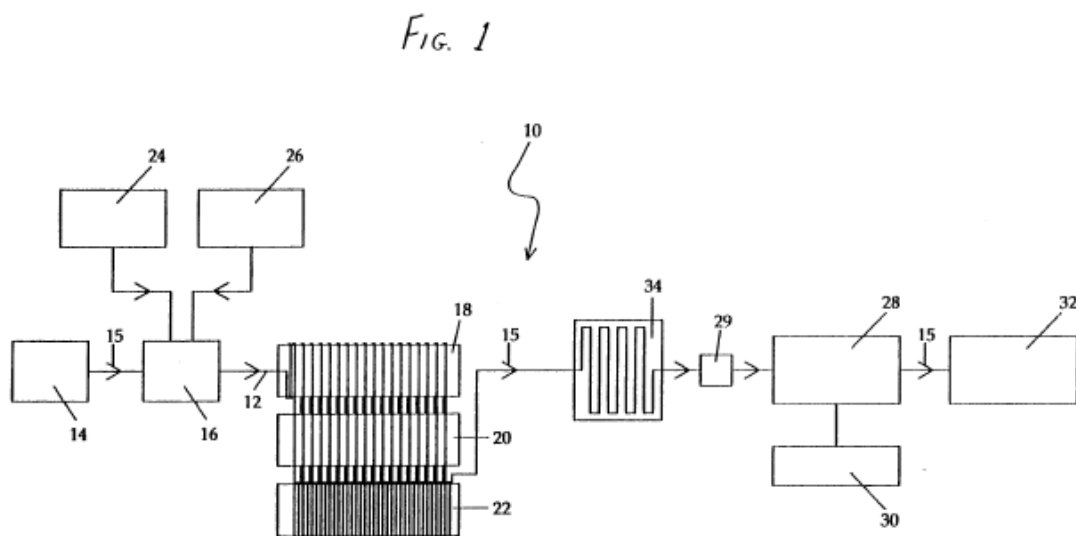
1618. It also would have been obvious to form at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by portioning the aqueous fluid with the flowing oil at the junction of the at least two channels based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1619. Claim 1 further recites: **“and providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.”**

1620. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes the conditions suitable for conducting a glucose reaction: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

1621. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus

and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR

was one type of reaction that could be conducted within droplets in a microfluidic system.

1622. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1623. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops

through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1624. It also would have been obvious to provide conditions suitable for the autocatalytic reaction in the at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1625. Claim 2 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1626. Claim 2 further recites: “**the at least one substrate molecule is a single biological molecule.**”

1627. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes droplets comprising biological molecules. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

1628. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation.

I incorporate my analysis with respect to ¶¶ 1157-1160, demonstrating how Quake discloses that the at least one substrate molecule is a single biological molecule.

1629. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566.

1630. It also would have been obvious that the at least one substrate molecule is a single biological molecule based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

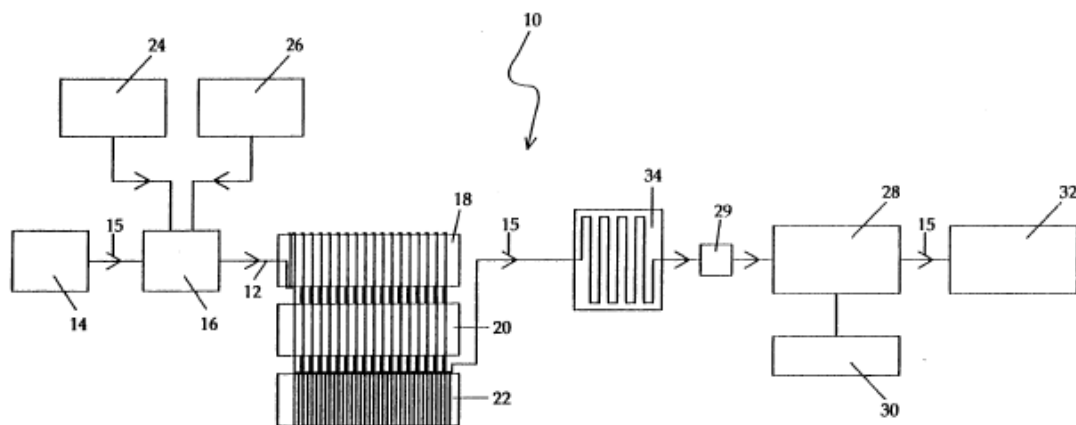
1631. Claim 3 of the '193 patent is dependent on claim 2. I incorporate by reference my analysis with respect to claims 1 and 2.

1632. Claim 3 further recites: “**the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.**”

1633. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the

amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1634. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1635. It also would have been obvious that the at least one substrate molecule is DNA

and the autocatalytic reaction is a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1636. It also would have been obvious that the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 4*

1637. Claim 4 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1638. Claim 4 further recites: “**the providing step includes heating.**”

1639. Seki satisfies this limitation. For example, Seki discloses that “[a] temperature of the microchip is adapted to be controlled by a temperature controller.” Seki at [0126].

1640. While it is my opinion that Seki discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by

heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1641. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1642. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1643. It also would have been obvious to provide heating to the microfluidic system based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 5*

1644. Claim 5 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1645. Claim 5 further recites: “**providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.**”

1646. Seki satisfies this limitation. For example, Seki discloses that “when a microchip involves a control mechanism for a trace quantity of liquid according to the second embodiment of the invention in a manner, for example, as described above, an analysis, a chemical reaction, or the like wherein a trace quantity of a sample is handled can be conducted. In this case, since the whole microchip is transparent, a variety of reactions of liquids introduced in the microchip can be easily observed.” Seki at [0143].

1647. While it is my opinion that Seki discloses providing a detector, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1187-1182, demonstrating how Quake discloses a detector.

1648. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1649. It also would have been obvious to provide a detector based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 6*

1650. Claim 6 of the '193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1651. Claim 6 further recites: “**the oil is fluorinated oil.**”

1652. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1653. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1654. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been

used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1655. It also would have been obvious to use a fluorinated oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 7*

1656. Claim 7 of the ’193 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1657. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1658. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1659. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as

therapeutic agents. *Id.* 2:20-58.

1660. It also would have been obvious to use a surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 8*

1661. Claim 8 of the '193 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1662. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1663. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1664. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1665. It also would have been obvious to use a fluorinated surfactant based on Seki in

light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and X.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 11*

1666. Claim 11 of the '193 patent is dependent on claim '1. I incorporate by reference my analysis with respect to claim 1.

1667. Claim 11 further recites: **“the at least one plug is substantially spherical in shape.”**

1668. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1203-1206, demonstrating how Quake discloses at least one plug that is substantially spherical in shape.

2. *Motivation to Combine and Reasonable Expectation of Success*

1669. A POSA would have seen compelling reasons to modify the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct different types of autocatalytic reactions (and in particular, PCR) in small volumes as taught by Corbett, Lagally, Burns (1996), or Wang. This is because the prior art clearly taught that reactions could be conducted within microfluidic droplets, and there were numerous advantages associated with these microfluidic droplet reactors. In particular, a POSA would have considered it obvious to modify the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR based on numerous teachings in the art, including Corbett, Lagally, and Burns (1996), which discussed small-scale and even on-chip PCR (*see, e.g.*, Burns (1996)). A

POSA would have had a reasonable expectation of success in so modifying, as evidenced by both the prior art and contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. Indeed, Quake itself describes both enzymatic reactions with biological molecules and PCR within microfluidic droplets. Quake at [0080] and [0170].

1670. A POSA would have been strongly motivated to perform autocatalytic reactions, including PCR, in the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki because doing so would have provided the substantial benefits known to be associated with microfluidic reactors. For example, Nisisako noted that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation, and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” *Treating liquid samples in droplet shape has the advantage that dead volume can be decreased.* Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is *likely to become increasingly important.*” Nisisako at 24 (emphasis added).

1671. A POSA would also prefer to carry out reactions in microfluidic droplets because its small dimensions allow for reduction of diffusion time for bimolecular reactions. Biomolecular reactions require two molecules to first encounter each other by diffusion or convection-enhanced diffusion. The reaction time and reaction yield for a given reactor are then determined by the diffusion time and then the kinetic time after the molecular encounter. By reducing the diffusion time, a micro-droplet reactor can significantly enhance the reaction yield. *See, e.g.,* Burns (2001) at 10. The reduction of the diffusion time also allows for careful analyses

of different kinetic times or kinetic rates, thus allowing for the selection or screening of chemical or biological catalysts. If such reactions involve thermal programming, the low thermal capacitance of droplets also allows very rapid temperature change, thus preventing undesirable by-products.

1672. For example, with protein crystallization, the reduction of diffusion time reduces exposure to non-ideal environments during the random-walk diffusion *See, e.g.*, (Chayen). A large number of small-volume droplets can also enhance selectivity. If the concentration of the droplets is higher than the concentration of interfering agents, the concentration of the interfering agents will be lower in the droplets. *See, e.g.*, Ferrance at 200.

1673. As another example, Lagally explained that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to *increase the speed of these assays* and to *reduce the amount of material and reagents needed*.” Lagally at 565 (emphasis added). Because PCR and other autocatalytic reactions rely on reagents that are often in limited supply—for example, sample DNA—the ability to reduce both the amount of material needed for the reaction to occur and the dead volume of the reaction would have been highly motivating. Ferrance similarly explained that “[t]he same advantages of *reduced time, sample, and reagents* brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions.” Ferrance at 192 (emphasis added). The modification of Corbett, Lagally, Burns (1996), or Wang to microfluidic dimensions would have reduced the amounts of reagents

used, which would in turn would decrease operating costs. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

1674. Curcio likewise taught that “[m]initurization of the fluidic system is beneficial in two ways: it *enhances the speed of thermal equilibration of the reaction mixture*, thus allowing increased flow velocities and faster PCR. Also *analyte volumes are reduced*, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level.” Curcio at 7 (emphasis added).

1675. Vogelstein additionally taught that microfluidic PCR enabled a sample to be diluted into thousands of discrete reaction volumes that each contained either one template PCR molecule or no DNA molecules. Vogelstein at 9236, 9239. A POSA would have found this advantageous because individual-template PCR reactions would have enabled the detection of relatively rare mutations, dislocations, and allelic imbalances. Vogelstein at 9236, 9239.

1676. Reduction in size of the reaction vessel also allows for precise quantification of, for example, nucleic acids and pathogens. As a single template nucleic acid or pathogen can be placed in a droplet, detection of successful PCR amplification in a given number of droplets allows for digital quantification of, for example, the number of template nucleic acid or pathogens present. For the same reasons, other types of patterns, including *irregular* expression of nucleic acids, could also be quantified. A POSA would have expected that the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform different types of autocatalytic reactions, including PCR, that would enable these applications.

1677. Further, conducting PCR in microfluidic droplets would reduce potential

contamination of the reaction, an issue that the prior art had recognized. *See, e.g.*, Corbett at 3:6-12 (“The most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of the assay.”).

1678. It was also well known that decreasing the scale of autocatalytic reactions, including PCR, to microfluidic levels provided the substantial advantage of making reactors portable. For example, Kopp explained that portable PCR microreactors could enable “[o]n-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Kopp at [1047]. Further, it was known that portable PCR reactors could aid physicians in the development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Kopp at [1047]. Thus, the prior art demonstrated that using the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to perform PCR (and other autocatalytic reactions) would have advantageously allowed PCR and other autocatalytic reactions to be performed in point of care diagnostic applications.

1679. Additionally, using the microfluidic reactors for PCR reactions would have substantially increased the tolerance of PCR reactions to primer non-specificity. As of the filing date, it was well known that PCR reactions suffered from the limitation that the primers were not always specific to the sequence of interest but rather could also bind to other sequences. Cha at 526. Because PCR amplification reactions are exponential in nature, PCR would often be ineffective where these other DNA fragments outnumbered the fragments of interest. *Id.* In such circumstances, the amplification products of the former would greatly exceed the amplification

of the latter. *Id.* By using multiple droplets, a POSA could reduce the chances of having an uncontaminated DNA template in a single reaction. *Id.* Further, a POSA could conduct exponential amplification of the template without having the intended amplification product compete with unintended amplification products. *Id.*

1680. Moreover, a POSA would have expected the combination of microfluidic droplet reactors and different types of autocatalytic reactions, including PCR, to be successful. For example, in 2001, Lagally et al. provided an overview of the evolution of continuous flow PCR microreactors:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR

chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Lagally at 565-570.

1681. In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct single-molecule DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

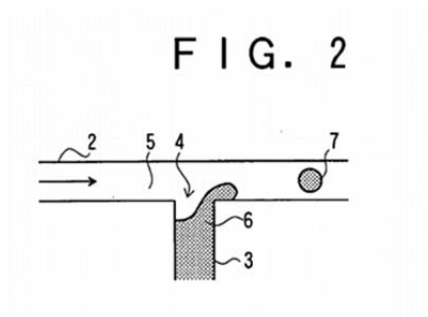
The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-

density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Lagally at 566-570. Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. Consistent with the observations in the prior art, a POSA would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR reactions or other autocatalytic reactions.

1682. The fact that several other groups simultaneously developed microfluidic systems that fall within the claims of the Ismagilov patents provides further evidence that a POSA would have both found the combinations described above obvious and would have had a reasonable expectation of success in so combining. For example, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. *See* Higuchi I-III.

1683. Higuchi I discloses “a process and apparatus for rapidly producing an emulsion and microcapsules in a simple manner.” Higuchi I at Abstract. As an example, Higuchi describes that “[a] process for producing an emulsion includes a step of ejecting a dispersion phase from a dispersion phase-feeding port toward a continuous phase flowing in a microchannel in such a manner that flows of the dispersion phase and the continuous phase cross each other, whereby microdroplets are formed by the shear force of the continuous phase and the size of the microdroplets is controlled.” Higuchi I at [0006]. This is illustrated by Figure 2 in Higuchi I, reproduced below:



Higuchi I at Fig. 2. In the text accompanying the figure, and corresponding with the numbers, Higuchi I describes that “[a] dispersion phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Abstract. The microfluidic droplet system Higuchi and his colleagues developed was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet systems to create droplets from continuously flowing streams of water and oil—and the use of those droplets to conduct reactions—was within the level of skill in the art as of the earliest effective priority date.

1684. As another example, Todd Thorsen (who co-authored the Thorsen reference discussed above) also developed a droplet reactor that falls within the claims of the Ismagilov patents. Thorsen Thesis at 94-108. The Thorsen Thesis describes the following microfluidic droplet reactor:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under

monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

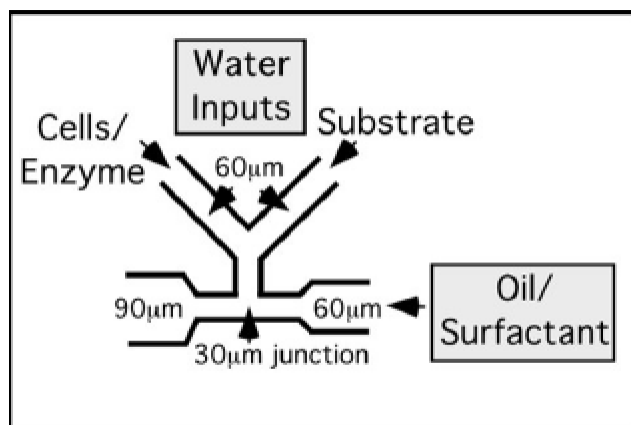


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

1685. The Thorsen thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002, suggesting that Thorsen’s work was performed before this date. The Thorsen Thesis was deposited with CalTech THESIS on December 2, 2002. Thorsen Thesis at 10X-000255686. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

1686. A POSA would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Schubert, or Krafft in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and

Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ramsey at 6:49-50 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). Krafft also noted that “the relatively low reactivity of fluorchemicals allows them to be combined with a wide variety of compounds without altering the properties of the incorporated agent.” Krafft at 2:27-30. For example, Curcio described that perfluorodecalin was utilized as a carrier fluid with small-volume PCR because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity [and thus, high biocompatibility] towards biomolecules.” Curcio at 9. Therefore, a POSA conducting a reaction with a biological molecule in microfluidic droplets, such as PCR and other types of autocatalytic reactions, would have used fluorinated oils and fluorinated surfactants with these microfluidic droplet systems. For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

1687. Further, fluorinated oil offers high immiscibility with water and low solubility of biomolecules. *See, e.g.*, Schubert at 97 (“Fluorinated compounds also offer the potential for biomedical applications. For example, . . . fluorinated alkanes are . . . chemically and biologically stable.”); *id.* (“Because fluorocarbons are insoluble in water, however, they are currently administered in the form of emulsions, the formation of which requires the use of surfactants.”). Unlike most mineral oils, fluorinated oil has a density higher than water. Gelest at

19. This higher density allows easy separation of aqueous droplets from the oil when the emulsion is collected off the substrate.

1688. The art had also already noted that fluorination was preferable for silicon-based microfluidic devices, which have a tendency to swell when exposed to hydrocarbon oils. *See* Quake at [0118] (emphasis added) (“**TEFLON [which contains fluorination] is particularly suitable for silicon elastomer (RTV) channels**, which are hydrophobic and advantageously do not absorb water, but *they may tend to swell when exposed to an oil phase.*”). As Quake noted, “[s]welling may alter channel dimensions and shape, and may even close off channels, or may affect the integrity of the chip, for example, by stressing the seal between the elastomer and a coverslip.” Quake at [0118]. This issue was also prevalent with PDMS, a silicon material that was commonly used to manufacture microfluidic substrates. *See* Quake at [0216] (emphasis added) (“In a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicon rubber or PolyDiMethylSiloxane (PDMS), **preferably PDMS.**”); ’407 patent at 16:59-61 (“Channels may be molded onto optically transparent silicon rubber or polydimethylsiloxane (PDMS), **preferably PDMS.**”).³² Unlike other organic oils, fluorinated oil does not cause polymer like PDMS to swell. Holtze at 1632 (“In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.”) (citing Lee). Therefore, a POSA would have been motivated to use fluorinated oils and surfactants to prevent swelling of the polymer substrate.

1689. Importantly, fluorinated oil is far less viscous than other oils, including mineral oils. *See generally* Gelest. Instead, fluorinated oil has a viscosity similar to water. *Id.* Using a

³² I note that this language in the Ismagilov patents was copied almost directly from Quake.

fluorinated oil with a microfluidic droplet device would thus allow high-frequency generation of droplets and parallel generation with multiple orifices. The prior art had already shown that high-throughput droplet generation was desirable. *See* Quake at [0079] (“This arrangement can be used to improve throughput or for successive sample enrichment, and can be adapted to provide a very high throughput to the microfluidic devices that exceeds the capacity permitted by conventional flow sorters.”); Quake at [0093] (“Monodisperse droplets may be particularly preferabl[e], e.g., in high throughput devices and other embodiments where it is desirable to generate droplets at high frequency.”). Further, the viscosity of fluorinated oil is insensitive to temperature, which is particular useful for DNA amplification reactions involving temperature changes. This of course includes PCR. Mullis at 9:55-60. For these reasons, a POSA would have been motivated to use fluorinated oil to achieve higher frequency droplet generation. Indeed, fluorinated oil has become the preferred carrier fluid for high-throughput aqueous droplet microfluidics. Autour at Section 4.1.

1690. As the prior art demonstrates, a POSA would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct autocatalytic reactions, including PCR, as taught by Corbett, Lagally, Burns (1996), or Wang.

XI. INVALIDITY OF THE '148 PATENT

A. Summary of the '148 Patent

1691. The '148 patent is entitled "Method of Performing PCR Reaction in Continuously Flowing Microfluidic Plugs." The abstract explains that the invention "provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid." '148 patent at Abstract.

1692. I understand that Plaintiffs are asserting claims 1-3 and 6-8 of the '148 patent. Of these claims, only claim 1 is independent. Claims 2, 3, 6, and 7 depend on claim 1. Claim 8 depends on claim 7.

1693. The '148 patent issued from Application No. 13/563,347, filed December 27, 2012 (the "'347 application"). The '347 application was a continuation of the '155 application (which issued as the '193 patent), which was a continuation of 12/777,099, filed on May 10, 2010, which was a continuation of application No. 10/765,718, filed on January 26, 2004, which itself was a continuation-in-part of application No. 10/434,970 (which issued as the '091 patent), filed on May 9, 2003.

1. *Priority*

1694. I understand that Bio-Rad asserts that claims 1, 2, 3, 6, 7, and 8 of the '148 patent were conceived of "no later than October 30, 2002," and relies on RI00106817–18, RI00111308, RI00111321, RI00111340–41, and RI00111690–1738 to support this assertion. Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 6. I disagree with Bio-Rad's assertions. The cited documents do not demonstrate the inventors had formed their minds the definite and permanent idea of a complete and operative invention as of the dates alleged.

1695. RI00111690-738, which Bio-Rad relies on to evidence conception of the asserted

claims, appears to be a grant application. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 30, 2002, or establish that the inventors had possession of every feature recited in the asserted claims as of that time. For example, all claims require “target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other,” and there is no discussion of fluids containing such molecules anywhere in RI00111690-738. Indeed, the only specific reference to DNA or RNA in RI00111690–1738 is the statement that “schemes can be readily constructed in which a single molecule of DNA, RNA, or a protein labeled with nanoparticles is detected visually via [an] autocatalytic pathway” after the molecule is “label[ed]” with metallic autocatalyst—*not* after that molecule undergoes a polymerase chain reaction. RI00111722–23. All claims similarly require “providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified,” and the only discussion of any “polymerase chain reaction” in RI00111690–738 is as a general example (along with silver halide photography) of a context in which an autocatalytic reaction may take place. RI00111719.

1696. RI00106817-18, RI00111308, RI00111321, and RI00111340-41, which Bio-Rad relies on to evidence conception of its asserted claims, appear to be entries in various lab notebooks. These documents do not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 20, 2002, or establish that the inventors had possession of every feature recited the asserted claims as of that time. As an initial matter, Bio-Rad has identified no evidence corroborating its apparent assertion that these entries were created on or before the dates written on these notebook pages (for

example, these notes were not witnessed or countersigned by a third party). Further, these documents do not establish that the inventors had possession of every feature recited in the asserted claims, or that every limitation of these claims was known to the inventor as of October 20, 2002. For example, all asserted claims require the formation of plugs from a fluid comprising “target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other,” and there is no discussion of such fluids in any of these notebook pages.

1697. I understand that Bio-Rad asserts that each of the asserted claims of the ’148 patent was reduced to practice no later than May 9, 2003, based on the filing of U.S. Patent Application No. 10/434,970 on that date. *See* Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 6. In my opinion, however, the ’970 application fails to contain sufficient written description to establish that the inventor had possession of the alleged inventions claimed in the ’148 patent, and fails to enable any other asserted claims of the ’148 patent. This is discussed in more detail below, for example, because all of the deficiencies identified in the specification of the ’148 patent are also present in the ’970 application.³³

1698. I understand that Bio-Rad has provided no evidence of the inventors’ diligence in reducing the alleged inventions of the ’148 patent to practice after Bio-Rad’s alleged dates of conception, and consequently that there is no evidence that any claim of the ’148 patent would be entitled to priority as of Bio-Rad’s alleged dates of conception, even if these dates were uncontested (which they are not). Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 5-6. Further, as set forth in **Exhibit 2**, I have reviewed

³³ Accordingly, the listed claims of the ’148 patent are also not entitled to claim priority to the ’927 provisional application.

various lab notebooks from Dr. Ismagilov's lab dated May 9, 2003. None of these notebooks suggest that any work was done to reduce the inventions claimed in the '148 patent to practice in the nearly seven months between the alleged date of conception (October 30, 2002) and the alleged date of reduction to practice (May 9, 2003). For example, none of these notebooks include experiments or work relating to conducting PCR is plugs.

1699. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception, diligence, or reduction to practice (and I understand that 10X's position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

B. Invalidity Overview

1700. As shown in further detail below, my opinions regarding the '148 patent include the following:

- All asserted claims are invalid under Section 112 for lack of proper written description, lack of enablement, and/or indefiniteness.
- All asserted claims are obvious in light of Quake under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Shaw Stewart under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Burns (2001) under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Nisisako under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Thorsen under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Seki under Section 103 (either alone or in combination with other references).

C. Invalidity Under 35 U.S.C. § 112

1701. As described in further detail below, it is my opinion that the asserted claims of the '148 patent are invalid under 35 U.S.C. § 112.

1. *Written Description*

1702. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '148 patent are invalid for lack of written description.

1703. The claims of '148 patent, for example, require “**providing conditions suitable for a polymerase-chain reaction.**” I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Bio-Rad appears to be taking the position that the “**providing conditions suitable for a polymerase-chain reaction**” is far broader than what was disclosed in the '148 patent. Based on Bio-Rad's 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,822,148 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 32-36; *see also* Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 22. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X “provides conditions suitable for polymerase chain reaction” by “utilize[ing] and enzymatic denaturation process” and “primers and a polymerase for replication.” Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 25. I have reviewed the '148 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. The specification describes a single DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain

reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’148 patent at 44:58-61; ’148 patent at 2:65-67. There is no mention in the ’148 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. Indeed, there is no indication that the inventors of the ’148 patent contemplated any DNA amplification reaction beyond the basic (and well-known) PCR reaction. Nor has Bio-Rad identified any disclosure in the ’148 patent specification that discloses other DNA amplification reactions.

1704. There is also, for example, no adequate description of performing a “**providing conditions suitable for a polymerase-chain reaction**” in plugs *outside of a substrate*, including, for example, a DNA amplification reaction outside of a substrate. I have reviewed the ’148 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. There is no indication that the inventors of the ’148 patent contemplated performing a DNA amplification reaction in plugs off the substrate. Nor has Bio-Rad identified any disclosure in the ’148 patent specification that discloses a DNA amplification reaction in plugs off the substrate.

1705. Bio-Rad has taken the following position:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and]

all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X's Interrogatory No. 3. As an initial matter, none of the identified reactions are DNA amplification reactions.

1706. Further, in each of the “embodiments in which droplets are captured in a capillary tube” (the “capillary tube embodiments”) identified by Bio-Rad, the droplets remain separated by carrier-fluid such that the risk of droplet coalescence is minimized.

1707. First, Bio-Rad cites the description of “a microfluidic device of the present system can include further include capillary tubing suitable for collecting plugs (“the capillary device”; FIG. 46). . . [where] [u]pon formation of plugs in the PDMS portion and their transfer into capillary tubing, the flow rates are stopped, the capillary tubing is disconnected from the PDMS portion and the ends are sealed by capillary wax.” ’148 patent at 58:47-49, 58:65-59:1. As shown in Figure 46, this “capillary device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

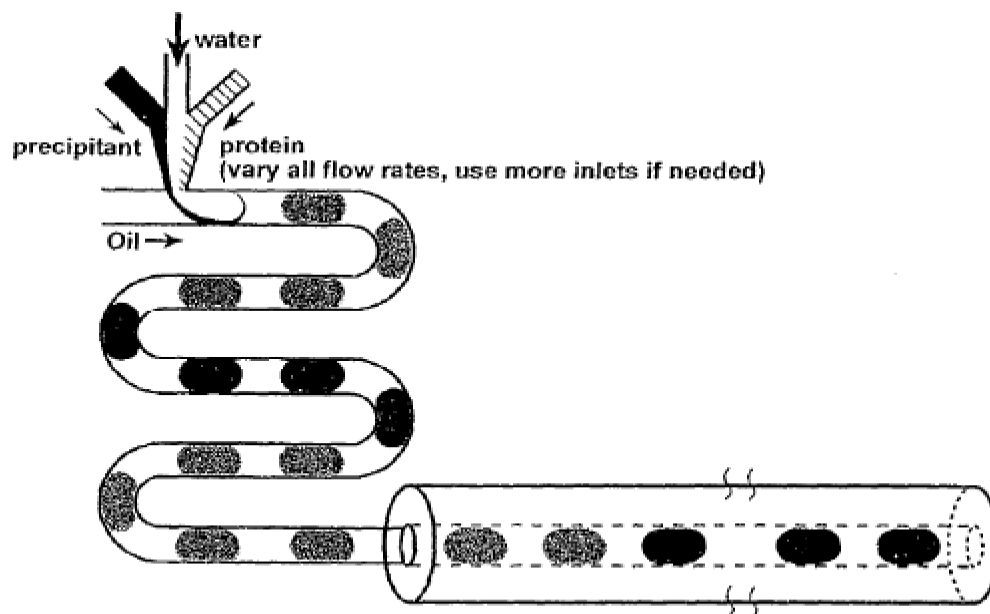


FIGURE 46

1708. Second, Bio-Rad cites Example 18 which states: “The capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator (18° C.). A lysozyme crystal appeared within an hour and was stable for at least 14 days without change of size or shape (FIG. 47A).” ’148 patent at 75:55-59. As shown in Figure 47A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

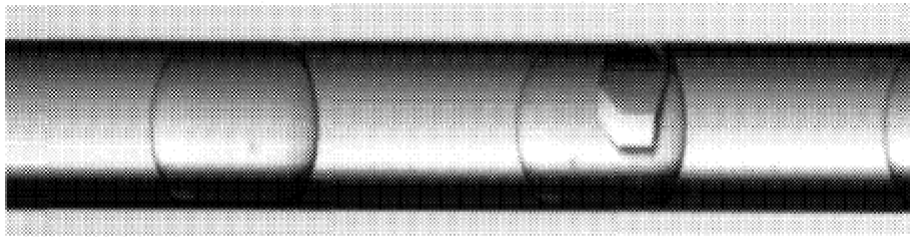


FIGURE 47A

1709. Third, Bio-Rad cites Example 19 which states: “The capillary was cut from the PDMS device, sealed by wax and stored in an incubator (18° C.). The thaumatin crystal appeared in 2-3 days and was stable for at least 45 days without size or shape change (FIG. 47B).” ’148 patent at 76:12-15. As shown in Figure 47B, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

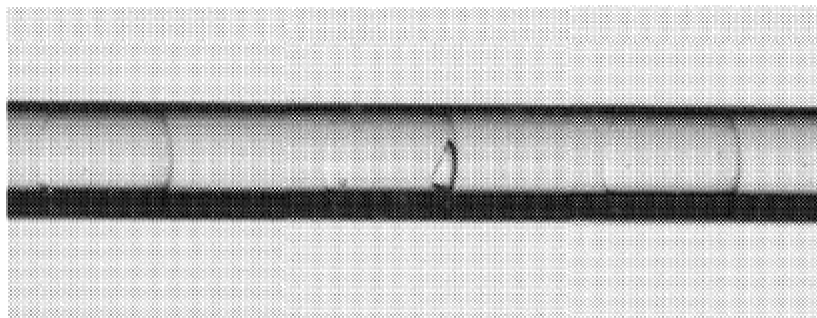


FIGURE 47B

1710. Fourth, Bio-Rad cites Example 20 which states: “After establishing alternating

aqueous droplet streams in the capillary, the flows were stopped, and the capillary was disconnected from the PDMS device, sealed with wax and stored in an incubator at 18° C.” ’148 patent at 76:61-64. As shown in Figure 50A, this “PDMS device” is configured such that the droplets remain separated by carrier-fluid and the risk of droplet coalescence is minimized:

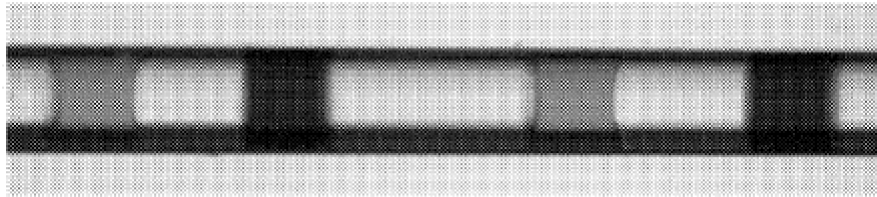


FIGURE 50A

1711. The “capillary tube embodiments” generally describe an extension of the microchannel that can be removed from the substrate while maintaining plug separation. These embodiments would not convey to a POSA that the inventors contemplated performing reactions in plugs outside of the substrate, for example in a well as performed by 10X. I understand that the image below is an image of droplets,

1712.

Due to a difference in oil and water density, oil will drain out of the emulsion such that the droplets are

closer together, increasing the potential of coalescence. The same is true for droplets collected using a micropipette or centrifuge tube. The capillary tube embodiments would not convey to a POSA that the investors had contemplated collection of droplets under these conditions. Further, as discussed below, Bio-Rad has not identified any teaching in the '148 patent that would convey to a POSA that the inventors had possession of a surfactant that would stabilize droplets and prevent droplet coalesce to allow for "providing conditions suitable for a polymerase-chain reaction" outside of the substrate, let alone a DNA amplification reaction outside of the substrate.

1713. In addition to the "capillary tube embodiments," Bio-Rad has cites to a portion of the specification as "*contemplat[ing]* collection of droplets and removal from the chip," First Supplemental Response to 10X's Interrogatory No. 3 (emphasis added):

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'148 patent at 17:16-23. As an initial matter, this section "contemplates" collecting "*solution*" not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in "a standard 1.5 ml centrifuge tube" or the "[c]ollection . . . using micropipettes"³⁴ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection, and subsequent DNA amplification off the substrate.

³⁴ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 ("Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes."); *see also* Quake at [0148].

1714. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. However, as discussed below, the surfactant depicted in Figure 24 would not stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets outside of the substrate.

1715. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927,³⁵ these applications lack adequate description of performing an “**providing conditions suitable for a polymerase-chain reaction**” including, for example, a DNA amplification reaction. Bio-Rad appears to be taking the position that “**providing conditions suitable for a polymerase-chain reaction**” is far broader than what was disclosed in the ’544 and ’927 applications. I have reviewed the ’544 and ’927 applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. The specifications of the ’544 and ’927 applications do not include a single reference to a DNA amplification reaction. There is no mention in the ’544 or ’927 applications of (for example) *any* DNA amplification reactions, let alone the details necessary to carry out said reactions. Nor has Bio-Rad identified any disclosure in the ’544 or ’927 specifications that discloses other DNA amplification reactions.

1716. The applications also lacks adequate description of “**providing conditions suitable for a polymerase-chain reaction**” outside of a substrate, including, for example, a DNA amplification outside of a substrate. I have reviewed the ’544 and ’927 applications, and

³⁵ I understand that Bio-Rad is not currently claiming that the claims of the ’148 patent are entitled to claim priority to these applications. Plaintiffs’ Corrected Response to Interrogatory No. 1 at 5.

they do not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the '544 and '927 applications that discloses a DNA amplification reaction in plugs off the substrate.

1717. Bio-Rad has taken the following position in its Response to 10X's Interrogatory No. 3:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be "up to several millimeters" in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as "PDMS"), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X's Interrogatory No. 3.

1718. But the '544 and '927 applications do not describe a single "embodiment in which droplets are captured in a capillary tube," let alone a DNA amplification reaction outside of the substrate.

1719. The specifications of the '544 and '927 applications state:

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'544 application at 28:22-26; '927 application at 24:14-23. Again, this section speaks to

collecting “*solution*” not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in “a standard 1.5 ml centrifuge tube” or the “[c]ollection . . . using micropipettes”³⁶ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalesce to allow such collection.

1720. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The ’544 and ’927 applications do not include this figure, or any related discussion. The ’544 and ’927 applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” ’927 application at 12:16-17; ’544 application at 12:19-13:5,³⁷ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids,

³⁶ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

³⁷ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 application at 10:31-11:15.³⁸ However, as discussed below, none of the surfactants described would stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets off the substrate.

1721. There is also, for example, no adequate description of **“providing conditions suitable for a polymerase-chain reaction.”** I understand the Court has construed “providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product” to mean “providing a set of physical and chemical conditions that allow the reaction to occur.” Claim Construction Order at

³⁸ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

1. Based on Plaintiffs' 4(c) disclosures, Bio-Rad contends that 10X "provid[es] conditions suitable," which includes "the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction," by, for example, "plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,822,148 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 32-36; *see also* Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 22. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X "provides conditions suitable for polymerase chain reaction" by "utilize[ing] and enzymatic denaturation process" and "primers and a polymerase for replication." Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 25. I have reviewed the '148 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the '148 patent that discloses providing the conditions suitable for a DNA amplification reaction to occur, let alone for a DNA amplification reaction to occur off the substrate.

1722. The specification describes a single DNA amplification reaction: "Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences." '148 patent at 44:58-61; '148 patent at 2:65-67. The specification does not set forth "a set of physical and chemical conditions that allow the [PCR] reaction to occur." There is no mention in the '148 patent of (for example) other DNA amplification reactions, let alone "a set of physical and chemical conditions that allow [said] reaction to occur."

1723. As another example, claims of the '148 patent require "**flowing aqueous fluid**

comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.”

Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provides the DNA and reagents under conditions in which they do not react” because “a DNA amplification reaction takes place once the droplets are transferred to a 96-well plate for thermal cycling, where the amplification of the DNA takes place.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 17-19; *see also* Appendix D to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 14. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4, Bio-Rad contends that 10X “[t]he reagents are provided under conditions in which the not not react,” because “[a] stimulus is provided to initiate the reaction.” Appendix D to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 15. “During this thermal cycling protocol, oligos which have been released as the gel bead fall apart prime off of the genome and do a low-level of copying.” *Id.* at 14, 24, 26. I have reviewed the ’148 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the ’148 patent that discloses “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.”

1724. The specification describes a single DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’148 patent at 44:58-61; ’148 patent at 2:65-67. The specification does not set forth any “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.”

2. *Enablement*

1725. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '148 patent are invalid for lack of enablement.

1726. Claims of the '148 patent, for example, require **“providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.”** I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X “provides conditions suitable” for the “PCR reaction in the plug” by “control[ling] [the] temperature to cycle the DNA amplification reaction” and the “target DNA or RNA is amplified” through a “PCR reaction.” Infringement of U.S. Patent No. 8,822,148 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 32-33; *see also* Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 22. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X “provides conditions suitable for polymerase chain reaction” by “utilize[ing] and enzymatic denaturation process” and “primers and a polymerase for replication.” Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 25. But the specification of the '148 patent does not enable the full scope of the limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. The claims purport to cover *all* DNA amplification reactions in plugs (whether known or unknown at the time of Ismagilov's alleged invention), but the specification includes a single reference to a DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” '148 patent at 44:58-

61. The specification does not include a working example of a PCR reaction in plugs. And there is no mention in the '148 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. The '148 patent fails to disclose, teach, or suggest how to conduct every "DNA amplification reaction," and particularly, the "DNA amplification reactions" allegedly performed by 10X,³⁹ within plugs.

. I understand that this technique was developed by 10X years after the priority date of the Ismagilov patents.

1727. As another example, claims of the '148 patent, for example, require "**providing conditions suitable for a polymerase-chain reaction.**" I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X "provides conditions suitable" for the "PCR reaction" by "the control of temperature to cycle the DNA amplification reaction" in its 10X GemCode™ platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,822,148 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 32-35; *see also* Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 22. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X "provides conditions suitable for polymerase chain reaction" by "utilize[ing] and enzymatic denaturation process" and "primers and a polymerase for replication." Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 25. The claims purport to cover *all* DNA amplifications in plugs (whether

³⁹ I have not been asked to provide, and have not formed an opinion on whether or not the reactions performed in 10X's products are "DNA amplification reactions."

known or unknown at the time of Ismagilov's alleged inventions), including DNA amplification reactions in plugs *outside of the substrate*. But the specification of the '148 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. The specification does not enable DNA amplification reactions in plugs *outside of the substrate*. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. Surfactants that would enable a POSA to conduct biological reactions within microfluidic droplets outside of the substrate, let alone DNA amplification reactions outside of the substrate, are not described in specification of the patent and were not even available as of the alleged priority date of the '148 patent. In fact, surfactants appropriate for this use were not developed or described until 2008—seven years after Ismagilov's alleged invention.

1728. As discussed above, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.”

1729. The surfactants disclosed in the Ismagilov patents would not stabilize droplets under these conditions.

1730. In order to conduct biological assays within microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, preventing coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Holtze at 1632.⁴⁰ “These requirements [were] particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluortelomer-tails (typically perfluorinated C₆ to C₁₀) . . . do not provide sufficient long-term emulsion stability.” *Id.*⁴¹ Even as of 2008, years after the priority date of the ’148 patent, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.*

⁴⁰ Holtze was authored by individuals from Harvard University, Universit`a del Salento, Lecce, Italy, and Raindance Technologies, Inc. Holtze at 1632.

⁴¹ When conducting biological assays in droplets, “it is attractive to use a fluorocarbon oil as the continuous phase” and accordingly, a fluorosurfactant to “ensur[e] that drops are stable.” Holtze at 1632.

1731. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. But none of the surfactants disclosed in the specification of the ’148 patent, including the surfactants disclosed in Figure 24, meet the requirements set forth above.

1732. As set forth in the specification, “FIG. 24 shows a reaction scheme that depicts examples of fluorinated surfactants that form monolayers that are: (a) resistant to protein adsorption; (b) positively charged; and (c) negatively charged. Fig. 24b shows a chemical structure of neutral surfactants charged by interactions with water by protonation of an amine or guanidinium group. FIG 24c shows a chemical structure of neutral surfactants charged by interactions with water deprotonation of a carboxylic acid group.” ’148 patent at 4:64-5:5

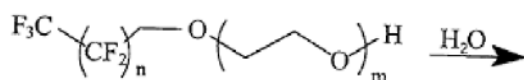


FIG. 24A

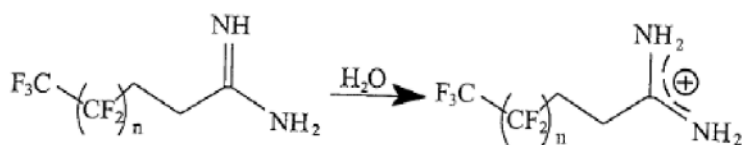


FIG. 24B

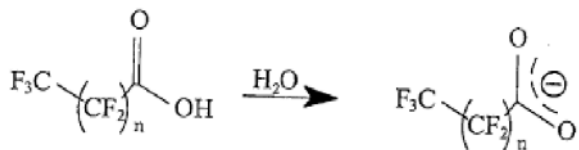


FIG. 24C

Fig. 24

1733. Specifically, Figure 24a “depicts a “fluorinated surfactants containing perfluoroalkyl chains [(red)] and an oligoethylene glycol head group [(blue)].” ’148 patent at 74:35-36.

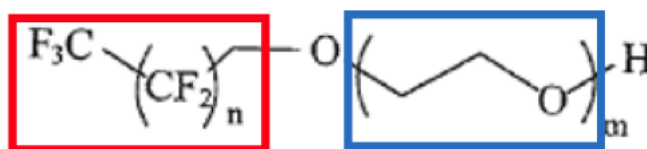
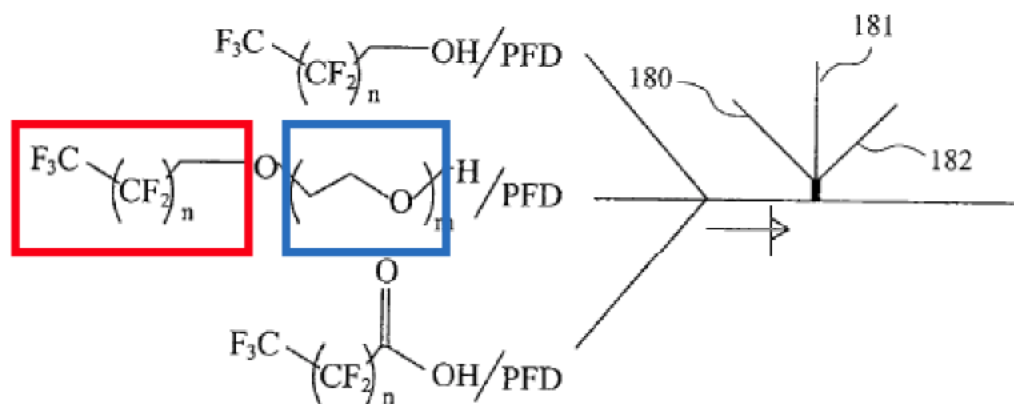


FIG. 24A

1734. The surfactant depicted in Figure 24a is commercially available under the trade name Zonyl. See '148 patent at 20:47-49 ("Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)"); '148 patent at 76:23-25 ("A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283.").

1735. Figure 18, depicts the same fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group. '148 patent at 57:10-12 ("In FIG. 18, plugs are formed in the presence of several solutions of surfactants that possess different functional groups (left side of the diagram)") (annotation added).

*Fig. 18*

1736. Unlike the ionic surfactants depicted in Figure 24b and 24c, the surfactant depicted in Figure 24a meets the second requirement set forth above. It will "produce a

biologically inert interior surface for the water drops.” As described in the specification of the ’148 patent: “[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.” ’148 patent at 35:63-66. Further, this OEG head group is non-ionic as required for biological assays. Holtze at 1632. But this surfactant does not meet the first requirement set forth above for performing biological assays in droplets. Specifically, it will not “provide stability to the drops, preventing coalescence.”

1737. The surfactant depicted in Figure 24a contains “a “perfluoroalkyl chains and an oligoethylene glycol head group.” ’148 patent at 74:35-36. A perfluoroalkyl chain (also referred to as a “perfluoroalkyl tail” of “fluorotelomer-tail”) is not sufficient to stabilize droplets outside of the substrate. As described by Holtze *et al.* “[s]urfactants with short fluorotelomer-tails” like the perfluoroalkyl chain depicted in Figure 24a, “do not provide sufficient long-term emulsion stability.” Holtze at 1632.

1738. I understand that Dr. Jeremy Agresti, Bio-Rad’s R&D Director and a co-author on Holtze *et al.*, confirmed this point. Dr. Agresti was questioned regarding the text copied below from Holtze *et al.*:

However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C₆ to C₁₀) have been used, but do not provide sufficient long-term emulsion stability. Fluorosurfactants with longer

fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632 (internal citations omitted).

1739. Dr. Agresti confirmed that these statements were accurate at the time they were written in 2008. Agresti Tr. 199:9-13(“Q. Do you believe that the statements that are made in the article that you just read, those portions that the article that you just read, do you believe those are accurate? A. Yeah, at the time for sure.”).

1740. Further in reference to the following statement in Holtze et al.: “Biological assays thus demand fluorosurfactants with non-ionic head groups; however, there are currently no such surfactants available,” Dr. Agresti confirmed that as of 2008 there were no “flourosurfactants with nonionic head groups that would stabilize and emulsion long term.” Agresti Tr. 202:2-13(“Q. And it was true that as of – as of the date of this article, which was 2008, that at least to your knowledge that there were no nonionic fluorosurfactants with nonionic head groups? A. That could stabilize an emulsion long term. We knew that there were fluoro surfactants with nonionic head groups. Q. [W]hat was not known was that there were fluoro surfactants with nonionic head groups that would stabilize an emulsion long term. A. Yes, that’s right.”). Dr. Agresti further confirmed that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, specifically Zonyl, “doesn’t stabilize droplets for PCR.” Agresti Tr. 203:10-19(Q. Are you familiar with a surfactant known as . . . ZONYL? A. Yes. Q. Has Bio-Rad used

that surfactant? A. I can't say. It's not in any product. As far as I know it's never been in any product. Q. Why not? A. As far as I know it doesn't stabilize droplets for PCR.”).

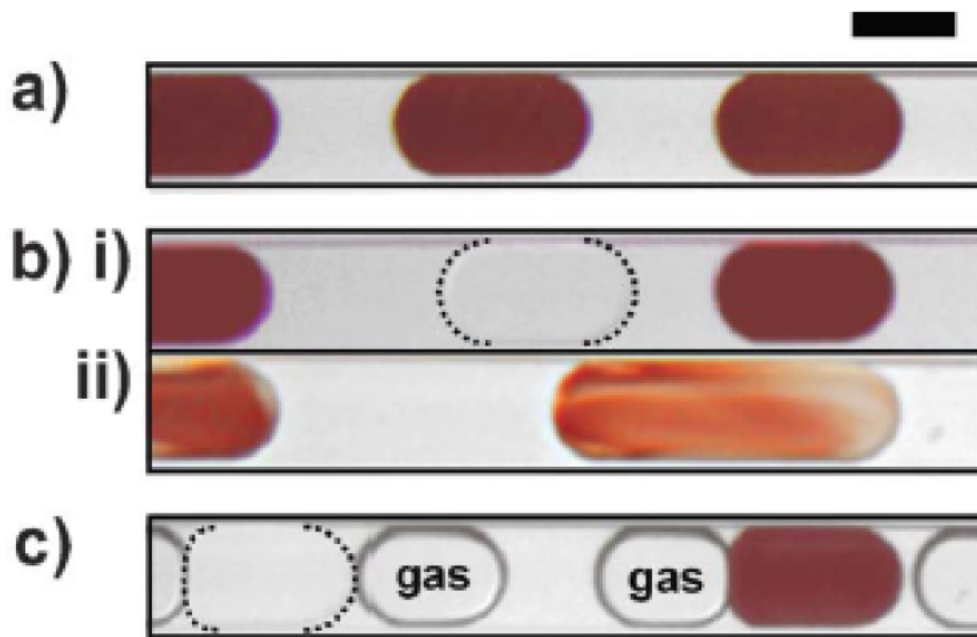
1741. I understand that named inventor of the '083 patent Mr. Lewis Spencer Roach, who testified that his “primary contribution” was to developing “fluorinated surfactant[s] [with] hydrophilic head group” Roach Tr. 26:21-23, also confirmed the point that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl would, not stabilize droplets long term. Mr. Roach testified that “other groups have done a lot of work on preventing coalescence using surfactants” but “I did not personally perform that research.” Roach Tr. 78:15-20. When asked whether “other groups” mean “other people in Dr. Ismagilov's lab,” Mr. Roach answered that he “believe[d] it was outside of Ismagilov's group.” Roach Tr. 78:21-79:2. Mr. Roach further testified that the “Rf-OEG surfactant is not optimized for preventing coalescence . . . [t]here are other hydrophilic head groups that are better at controlling adsorption than a simple oligo (ethylene glycol) head group. I think other people have made these.” Roach Tr. at 79:3-12.⁴² Mr. Roach later confirmed that the “other people” he was referring to were Holtze et al. in 2008. Roach Tr. 80:4-11 (“A. I believe [Exhibit 129 (Holtze et al.)] is what I was just referring to, that other groups had optimized surfactants to – give me just a second. I want to read the conclusions in this paper here. Q. Certainly. A. Yes. This is where I was discussing other groups that have optimized surfactants to prevent coalescence or merging of plugs.”).

1742.

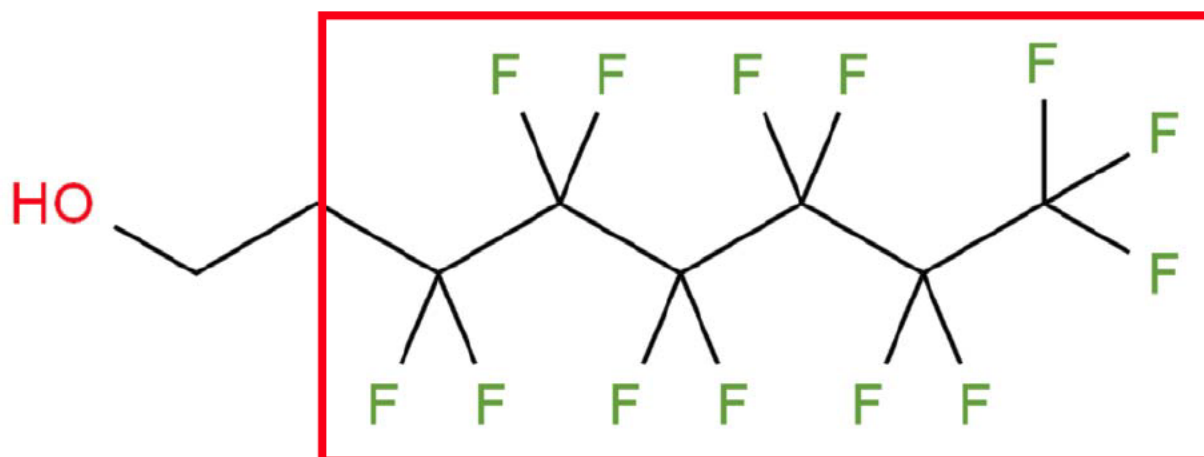
⁴² The “Rf-OEG” surfactant is a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group. *See* Roach Depo. Ex. 127 (“Perfluorinated-tail, oligoethylene glycol derivatized molecules (Rf-OEG) were selected as a neutral and hypothetically biocompatible surfactants.”); Roach Tr. 51:22-23 (“A. I synthesized the surfactant described in [Exhibit 127], particularly the Rf-OEG surfactant.”).

1743. I agree with Agresti's, Roach's, and Hindson's statements regarding fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl. Such surfactants would not provide stability to drops and prevent coalescence to allow for DNA amplification reactions in microfluidic droplet off-chip.

1744. Dr. Ismagilov himself recognized the potential for coalescence, even between plugs within the substrate. As explained by Dr. Ismagilov, "[d]uring flow, plugs with different chemical composition may move relative to the carrier fluid at different rates and thus move relative to one another allowing adjacent plugs to coalesce (Fig. 2(b))." Adamson at 1181.



1745. Figure 2b above depicts plug coalescence between “[p]lugs of distinct chemical composition.” Adamson at 1181. The carrier fluid is “FC-3283 10:1 PFO (v/v) throughout.” Adamson at 1181. FC-3283 is a fluorinated oil. PFO or 1H,1H,2H,2H-perfluorooctanol is a fluorinated surfactant. The chemical formulation of PFO is depicted below:

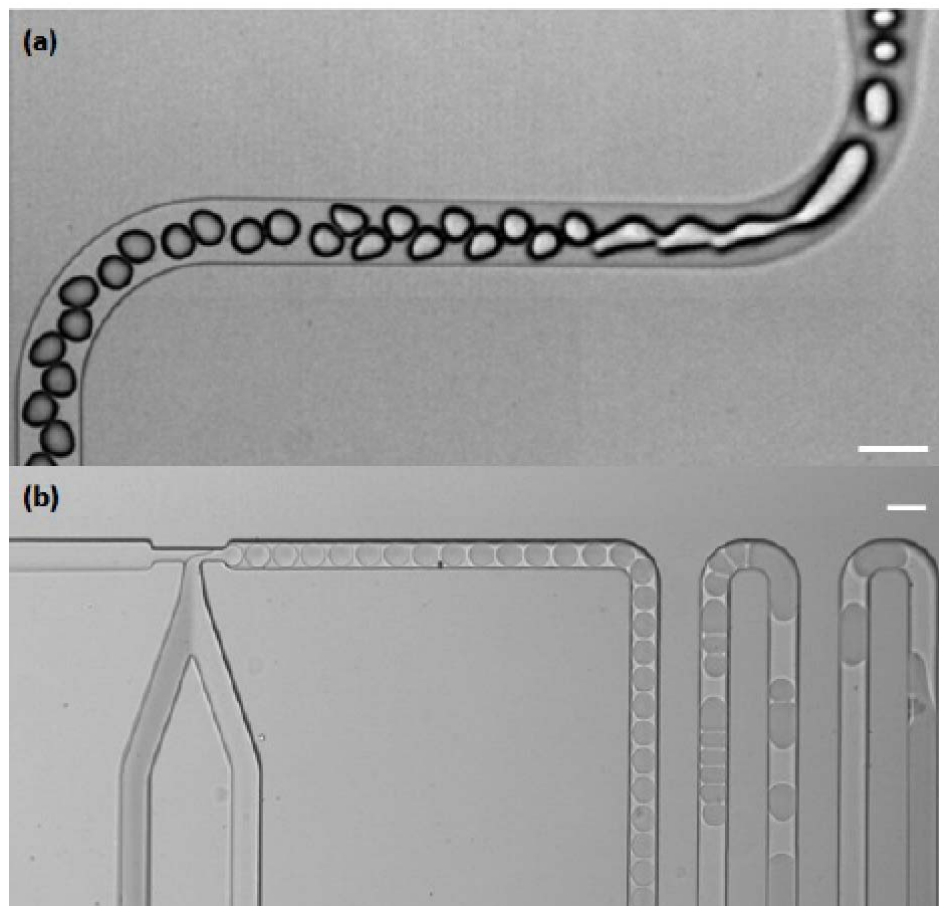


1746. Like Zonyl, PFO contains a short perfluoroalkyl tail (red).

1747. As explained by Dr. Ismagilov, this surfactant was insufficient to prevent

coalescence even within the substrate. *See* Adamson at 1181. Instead “[t]o prevent coalescence, gas bubbles [were] introduced as spacers between plugs to (1) minimize the relative motion of plugs and (2) to act as a physical barrier to prevent the coalescence of adjacent plugs during flow and splitting.” Adamson at 1181.

1748. This potential for droplet coalescence was later described as “[u]ncontrolled.” “Cho Thesis” at Fig. 3.1. This thesis explained: “Perfluorodecalin and 1H,1H,2H,2H-perfluoro-1-octanol combination was used for studying protein crystallization by Ismagilov and his coworkers. The perfluorinated oil and surfactants are advantageous for microdroplet based biochemical applications as they are lipophobic, inert, insoluble in water and compatible with many biochemical molecules. Unfortunately, droplets in perfluorodecalin oil with 1H,1H,2H,2H-perfluoro-1-octanol were not stable and merged with each other under pressure (Figure 3.1(b)).” Cho Thesis at 51. The “[d]roplet generation and uncontrolled coalescence of droplets in perfluorodecalin with 5% v/v 1H, 1H, 2H, 2H-perfluoro-1-octanol” observed is depicted in (b) below:



1749. “Fluorosurfactants with longer fluorocarbon tails” are required for “long-term stabilization” to perform biological assays. Holtze at 1632. As further described by Holtze et al., as of 2008, no such surfactant existed in 2008:

However, the only available PFPE-based surfactants have ionic headgroups, e.g. poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632. Holtze et al. disclose examples of fluorinated surfactants meeting the two requirements set forth above. These surfactants comprise non-ionic polyethylene glycol head

groups and perfluorinated polyether tails. Holtze at 1; Figure 2.

1750.

1751. , I understand that RainDance's droplet products utilize a "biocompatible surfactant, PEG-PFPE block copolymer." Plaintiffs' First and Second Supplemental Response to Interrogatory No. 5. Bio-Rad's droplet products utilize "Krytox K225 (0.58mM) + perfluorodecanol (0.625 mM) or the BRDG3 triblock fluorosurfactant." Plaintiffs' Third and Fourth Supplemental Response to Interrogatory No. 5.

1752. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927, the specifications of the '544 and '927 applications do not enable a person of skill in the art to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate without undue experimentation. Surfactants necessary to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate are not described in specification of the '544 or '927 applications and were not even available as of the filing date of the '544 or '927 applications.

1753. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The ’544 and ’927 applications do not include this figure, or any related discussion. The ’544 and ’927 applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” ’927 application at 12:16-17; ’544 application at 12:19-13:5,⁴³ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

⁴³ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

'544 application at 12:19-13:3; '927 application at 10:31-11:15.⁴⁴ In the context of conducting biological assays in microfluidic droplets outside of the substrate, each of the surfactants listed—excluding “fluorinated oil” discussed separately below—would be considered an aqueous soluble surfactant by a POSA, meaning they are introduced in the aqueous phase instead of the oil phase. To conduct biological assays in microfluidic droplets outside of a substrate, a POSA would understand that a continuous phase comprised of a fluorinated oil is preferred, if not necessary. Holtze at 1632. The listed surfactants are non-fluorinated and as such are not soluble in fluorinated oil. Therefore, to use one of the listed surfactants in a system comprising a fluorinated oil, the surfactant needs to be introduced into the aqueous phase. However, when present in the aqueous phase these surfactants would be disruptive to emulsion stability. The hydrophobic portions of these surfactant molecules cause them to populate the aqueous-fluorinated oil droplet boundary, displacing any stabilizing fluorinated surfactant molecules present. This process leads to droplet coalescence rather than stabilization.

1754. “Fluorinated oil” while soluble in fluorinated oil, also would not stabilize droplets to conduct biological assays in microfluidic droplets off of the substrate. Holtze at 1632 (“[I]t is

⁴⁴ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopahnitrate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

attractive to use a fluorocarbon oil as the continuous phase However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable.”).

1755. As another example, claims of the ’148 patent, require “**providing conditions suitable for a polymerase-chain reaction.**” I understand the Court has construed “**providing conditions suitable for a polymerase-chain reaction.**” to mean “providing a set of physical and chemical conditions that allow the polymerase-chain reaction to occur.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provides conditions suitable” for the “PCR reaction” by “the control of temperature to cycle the DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 32-35; *see also* Appendix D to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 22. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4, Bio-Rad contends that 10X “provides conditions suitable for polymerase chain reaction” by “utilize[ing] and enzymatic denaturation process” and “primers and a polymerase for replication.” Appendix D to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 25. The claims purport to cover providing conditions suitable for *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov’s alleged inventions), including conditions suitable for DNA amplification reactions in plugs *outside of the substrate*. But the specification of the ’148 patent does not enable the full scope of this limitation, at least under Bio-Rad’s actual and/or apparent application of the claims, without undue experimentation. The ’148 patent fails to disclose, teach, or suggest how to provide all conditions suitable to conduct a PCR reaction.

1756. Bio-Rad has taken the position that “the patents-in-suit disclose a comprehensive toolkit for conducting reactions in chemical droplets.” First Supplemental Response to 10X’s Interrogatory No. 3. Specifically, Bio-Rad has taken the position that “the patents-in-suit teach precisely: (1) the types of fluorinated oils and surfactants that have been used throughout the industry for this purpose; (2) the types of microfluidic devices that have been used for this purpose; and (3) the ability to precisely control the composition of droplets so that DNA amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, Bio-Rad provides no explanation for its assertions. As explained above, “the types of fluorinated oils or surfactants” described in the ’148 patent have *not* “been used throughout the industry for this purpose.” Instead, as explained above, each of 10X, RainDance, and Bio-Rad utilize surfactants with non-ionic polyethylene glycol head groups and a perfluorinated polyether tail, or tails. Further, the ’148 specification does not teach “the ability to precisely control the composition of droplets so that DNA amplification reactions can be initiated.” First Supplemental Response to 10X’s Interrogatory No. 3. The ’148 patent does not describe the “composition of droplets” necessary to conduct PCR.

1757. As another example, claims of the ’148 patent require “**conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.**” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X provides “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other” because “after the droplets come off the instrument, the reagents inside the droplets do not react. Rather, a DNA amplification reaction takes place once the droplets are transferred to a 96-well plate for thermal cycling.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 8, 18; *see also* Appendix D to Plaintiffs’

Supplemental Response to Interrogatory No. 4 at 14. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4, Bio-Rad contends that 10X "[t]he reagents are provided under conditions in which they do not react," because "[a] stimulus is provided to initiate the reaction." Appendix D to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 15. "During this thermal cycling protocol, oligos which have been released as the gel bead fall[s] apart prime off of the genome and do a low-level of copying." *Id.* at 14. But the specification of the '148 patent does not enable the full scope of the limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. For example, the specification of the '148 patent does not teach the use of a dissolvable gel bead to separate reagents contained within the same droplet, and accordingly prohibit a reaction with these reagents until the gel bead has dissolved.

1758. As another example, the claims of the '148 patent require a plurality of plugs **"each having a substantially uniform size of about 200 μm or less."** The specification of the '148 patent does not enable the full scope of this limitation. Bio-Rad has taken the position that "the specification includes a detailed disclosure of "Methods of Forming Plugs" which it contends "teaches how to control plug size." First Supplemental Response to 10X's Interrogatory No. 3. But the claims of the '148 patent fail to identify an express lower bound for plug size. The specification certainly does not enable one skilled in the art to form a plurality of plugs without *any* lower size limitation. For example, the '148 patent fails to disclose, teach, or suggest how to form a plurality of plugs that are 0.000000001 μm . I likewise understand that Bio-Rad has never identified or alleged any lower bound for plug size during that claim construction phase of the case or otherwise. In my experience it is difficult to generate uniform size droplets smaller than 10 μm using a T-junction microfluidic device like those described in the '148 patent.

1759. As another example, the claims of the '148 patent require a **“providing a microfluidic system comprising one or more channels.”** But the specification of the '148 patent does not enable the full scope of this limitation. Bio-Rad has taken the position that “[t]he specification of the '148 patent, however includes countless examples describing microfluidic systems with one more channels, all of which are shown in the Figures.” First Supplemental Response to 10X’s Interrogatory No. 3. But the specification of the '148 patent does not describe a single microfluidic system comprising *only* one channel, or teach a POSA how to “form plugs” in a microfluidic system comprising only one channel. Each of the microfluidic systems described in the specification contain *two* or more channels.

3. *Indefiniteness*

1760. It is my opinion that the claims of the '148 patent are invalid as indefinite because the '148 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention.

1761. Each claim of the '148 patent purport to cover **“providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.”** I understand the Court has construed “providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.” to mean “providing a set of physical and chemical conditions that allow the polymerase chain reaction to occur.” Claim Construction Order at 1. But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “a set of physical and chemical conditions that allow the reaction to occur.” For example, the specification does not inform, with reasonable certainty which “set of physical and chemical conditions” would allow a PCR reaction to occur. Without an adequate description of what constitutes “conditions suitable” for the reaction between the biological molecule and the

reagent, a POSA could not know whether he or she was practicing the claims.

1762. As another example, each claims of the '148 patent purport to cover “**under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.**” But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.” There is, for example, no adequate description of what constitutes the “conditions,” which could include a multitude of factors. Without an adequate description of what constitutes these “conditions,” an artisan could not know whether he or she was practicing the claims.

D. Invalidity Based on Prior Art

1. Obviousness

(a) Invalidity Based on Quake

1763. It is my opinion that Quake discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the '148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) Claim 1

1764. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

1765. Quake satisfies this limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

1766. Quake discloses that the microfluidic devices described contain at least two channels having at least one junction. For example, Quake states that “[t]he devices and methods of the invention comprise *a main channel*, through which a pressurized stream of oil is passed, and *at least one sample inlet channel*, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0068] (“The main channel is typically in fluid communication with an inlet channel or inlet region, which permits the flow of molecules, cells or virions into the main channel.”).

1767. Figure 16A in Quake also illustrates this limitation. Figure 16A is reproduced below:

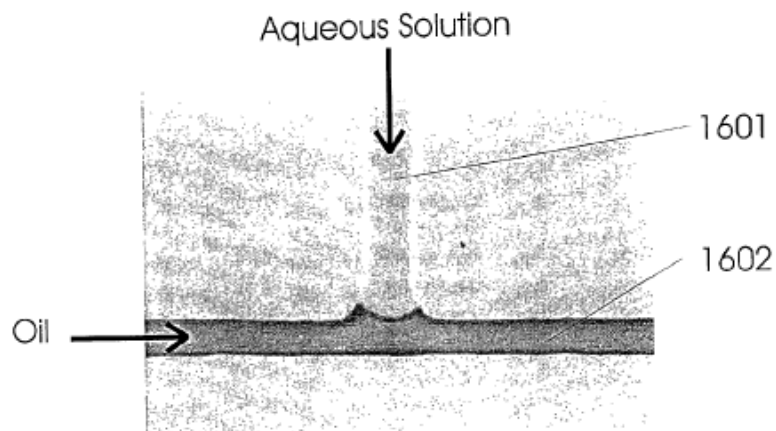


FIG. 16A

1768. Claim 1 further recites: “**providing within the one or more channels a continuously carrier fluid comprising an oil.**”

1769. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of

aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device*.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

1770. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (emphasis added) (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (*preferably a non-polar fluid such as decane or other oil*) in the main channel.”).

1771. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane

and hexadecane.” Quake at [0300].

1772. Quake describes that the “force and direction” of the flow of carrier fluid oil “can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels).” Quake at [0125].

1773. Quake also made clear that the “flow” of the oil was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and *a flowing stream of an immiscible fluid (e.g., decane)* it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

1774. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

1775. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, *through which a pressurized stream of aqueous solution is passed.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows

through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

1776. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device *and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.* The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample.*” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the *biological material may be, e.g., molecules* (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added). Quake also disclosed that the “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR.*” Quake at [0080] (emphasis added). As the ’148 patent explains, PCR is a type of autocatalytic reaction. *See* ’148 patent at 44:58-61 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”). Because double-stranded DNA is a highly stable molecule that does not react unless denatured (i.e., unwinds into single-stranded DNA), and denaturing of double-stranded DNA takes place in the presence of high temperatures or chemicals, a POSA would have understood that in the absence of high temperatures or

chemicals, the double-stranded would not have reacted. Mullis at 9:55-60.

1777. Quake also made clear that the “flow” of the aqueous fluid was continuous. For example, during prosecution of his patent application, Quake himself characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining *a flowing stream of an aqueous solution* and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15 (emphasis added).

1778. While it is my opinion that Quake discloses a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for

which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow of reaction mixture were not reacting with each other.

1779. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was

introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

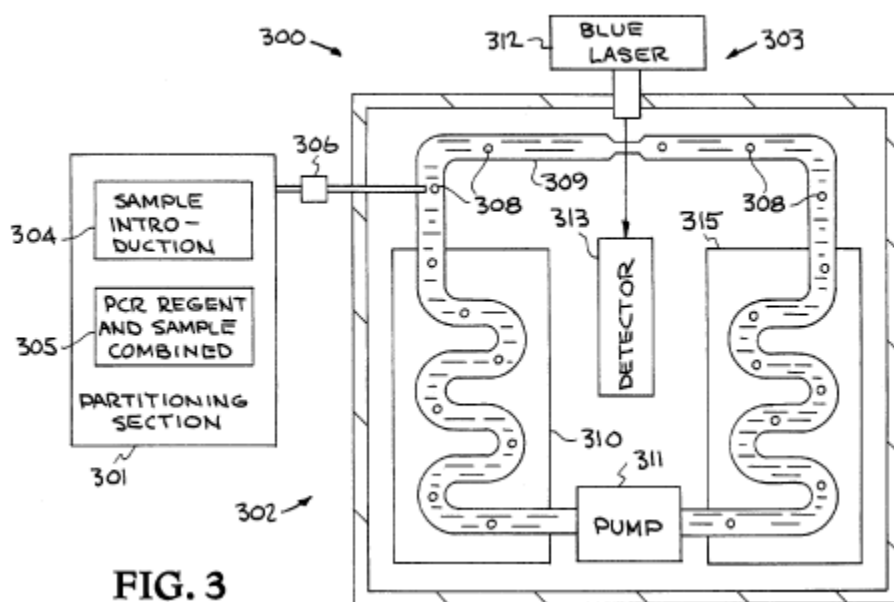
1780. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1781. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

1782. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and

the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.”

Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1783. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Id. at 7:47-50.

1784. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1785. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said**

carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.”

1786. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel*, e.g., at an angle that is perpendicular to the stream of oil. *By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream thereby forming droplets.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”).

1787. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” Quake at [0020] (emphasis added).

1788. Quake also made clear that his patent application described the forming of

droplets by partitioning aqueous fluid with carrier fluid. For example, during prosecution of his patent application, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15.

1789. Claim 1 further recites: **“each [plug] having a substantially uniform size of about 200μm or less.”**

1790. Quake satisfies this limitation. For example, Quake explains that “[f]or particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are preferably rounded, with a diameter between 2 and 100 microns, preferably about 60 microns, and more preferably about 30 microns at the crossflow area or droplet extrusion region . . . Similarly, the volume of the detection region in an analysis device is typically in the range of between about 10 femtoliters (fl) and 5000 fl, preferably about 40 or 50 fl to about 1000 or 2000 fl, most preferably on the order of about 200 fl. In preferred embodiments, the channels of the device, and particularly the channels of the inlet connecting to a droplet extrusion region, are between about 2 and 50 microns, most preferably about 30 microns.” Quake at [0091].

1791. Quake also provides a formula that can be used to calculate droplet size:

The size of a droplet in a micro fluidic device of this invention may be provided by the equation:

$$r = \frac{\sigma}{\eta \epsilon}$$

where r is the final droplet radius in a main channel. η , the viscosity of the continuous phase (e.g., the oil-surfactant phase in the above exemplary devices)

and σ , the interfacial tension, may be obtained from values available in the art for the particular fluids used (see, for example, CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Fla., 2000). ϵ , which denotes the shear rate, may be provided by the formula

$$\epsilon = \frac{2}{y_0} v,$$

where v is the velocity of the dispersed phase fluid (i.e., the droplets) and may be readily calibrated to the input pressures for a particular microfluidic device. Y_0 denotes the radius of the inlet channel at the droplet extrusion region (i.e., the radius of the tapered channel 1606 in FIG. 16B).

Quake at [0308]-[0310].

1792. Claim 1 further recites: “**wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.**”

1793. Quake satisfies this limitation. For example, Quake describes that “[t]he concentration (i.e., number) of molecules, cells or virions in a droplet can influence sorting efficiently and therefore is preferably optimized. In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” Quake at [0120].

1794. Indeed, during prosecution of the ’148 patent, applicants pointed to the following disclosure in the ’148 patent as providing support for the “Poisson distribution” limitation:

The concentration of reagents in a plug can be varied. In one embodiment according to the invention, the reagent concentration may be adjusted to be dilute enough that most of the plugs contain no more than a single molecule or particle, with only a small statistical chance that a plug will contain two or more molecules or particles. In other embodiments, the reagent concentration in the plug-fluid is

adjusted to concentrate enough that the amount of reaction product can be maximized.

See '148 Prosecution History, Amendment and Remarks, dated February 14, 2014 (RDTX00020229-38) at 4-5 (emphasis added). This disclosure is almost exactly the same as the disclosure in Quake. Therefore, though Quake does not explicitly name the Poisson distribution, a POSA would have understood that this description referred to a Poisson distribution.

1795. While it is my opinion that Quake discloses a Poisson distribution of target DNA or RNA in plugs, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

1796. It also would have been obvious to have a Poisson distribution of target DNA or RNA in plugs based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1797. Claim 1 further recites: “**and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule.**”

1798. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’

or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules (for example, polynucleotides*, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles, or mixtures thereof.” Quake at [0021] (emphasis added).

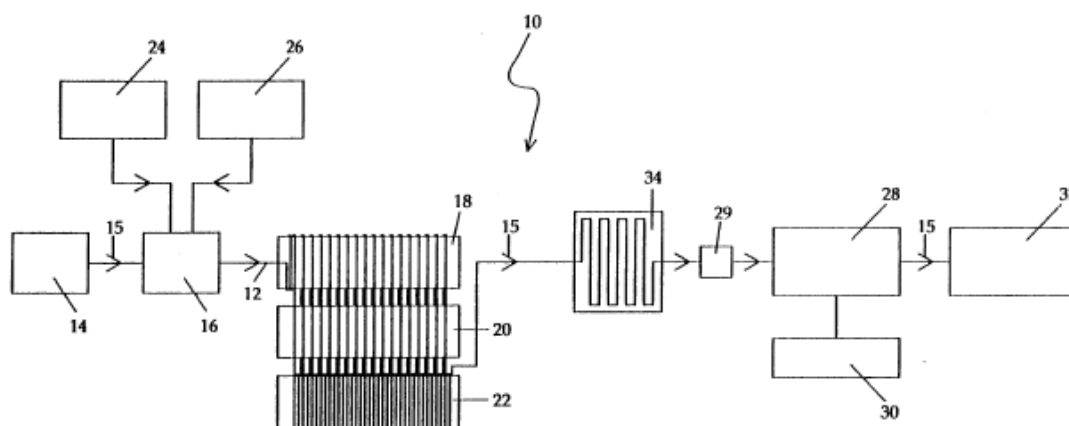
1799. Quake also makes clear that “[p]referably, *each droplet of this multi-phase mixture encapsulates a single particle*. The droplets are trapped and their boundaries are defined by channel walls, and therefore they do not diffuse and/or mix. Thus, *individual particles or molecules can be separately compartmentalized inside individual droplets*.” Quake at [0012] (emphasis added); *see also* Quake at [0015] (“In preferred embodiments, the second fluid includes a biological sample that comprises one or more molecules, cells, virions or particles. In exemplary embodiments for detecting and sorting droplet contents, the droplets of the second fluid each contains, on average’s [sic], no more than one particle. For example, in preferred embodiments where the biological material comprises viral particles, each droplet preferably contains, on average, no more than one viral particle.”).

1800. Quake also discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

1801. While it is my opinion that Quake discloses a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an

apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1802. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a

microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1803. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels

using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1804. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1805. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

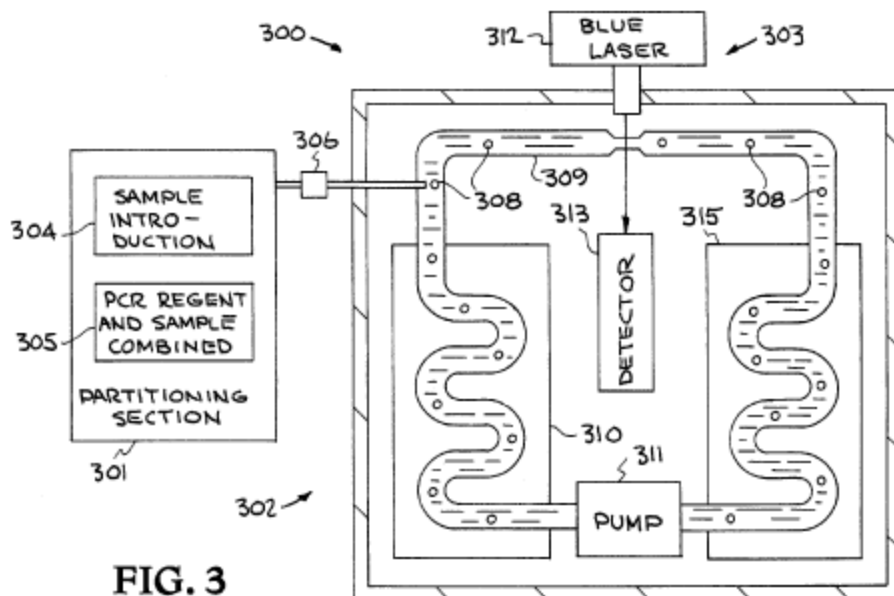


FIG. 3

1806. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:34-36.

1807. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

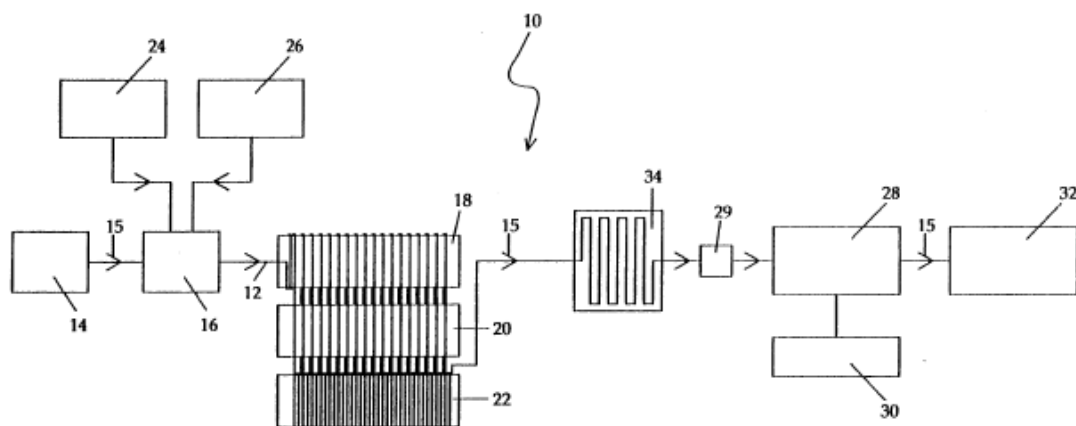
1808. Claim 1 further recites: “**and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.**”

1809. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry

on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

1810. While it is my opinion that Quake discloses providing conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1811. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying

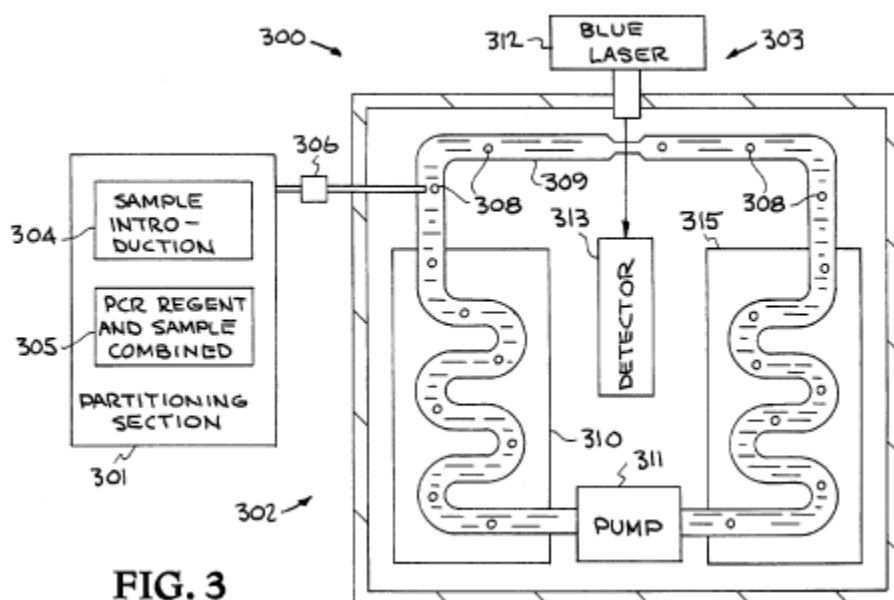
vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1812. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1813. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, *ampliTaq* polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA

would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1814. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1815. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped

through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50.

1816. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1817. Claim 2 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1818. Claim 2 further recites: **“the step of providing conditions includes heating.”**

1819. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Quake at [0080]. A person of skill in the art would have known that the PCR reaction required cycles of heating and cooling. Mullis at 9:55-60.

1820. While it is my opinion that Quake discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1821. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1822. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based

pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1823. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1824. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

1825. It also would have been obvious to provide heating to the microfluidic system based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

1826. Claim 3 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1827. Claim 3 further recites: “**providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.**”

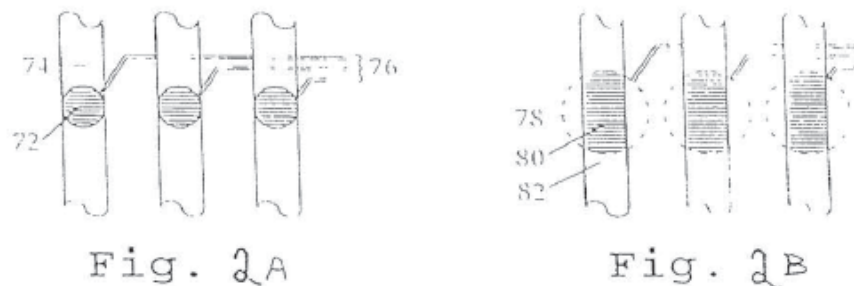
1828. Quake satisfies this limitation. For example, Quake describes that the microfluidic “device of the invention may also comprise a *detection region* which is within or coincident with at least a portion of the main channel at or *downstream of the droplet extrusion region*. The device may also have a *detector, preferably an optical detector such as a microscope*, associated with the detection region.” Quake at [0016] (emphasis added). Quake’s definition of “detection region” is also illustrative:

A “detection region” is a location within the chip, typically within the main channel where molecules, cells or virions to be identified, measured or sorted on the basis of a predetermined characteristic. In a preferred embodiment, molecules, cells or virions are examined one at a time, and the characteristic is detected or measured optically, for example, by testing for the presence or amount of a reporter. For example, *the detection region is in communication with one or more microscopes, diodes, light stimulating devices, (e.g., lasers), photo multiplier tubes, and processors (e.g., computers and software), and combinations thereof, which cooperate to detect a signal representative of a*

characteristic, marker, or reporter, and to determine and direct the measurement or the sorting action at the discrimination region.

Quake at [0069] (emphasis added).

1829. Quake also provides diagrams of a detector and detection region. Quake describes that “FIG. 2A shows one embodiment of a detection region used in a sorting device, having an integrated photodiode detector; FIG. 2B shows another embodiment of a detection region, having an integrated photodiode detector, and providing a larger detection volume than the embodiment of FIG. 2A.” Quake at [0025]. Figures 2A and 2B are reproduced below:



Quake at Figs. 2A and 2B.

1830. Indeed, most of the language in Quake describing detectors is almost exactly copied in Ismagilov’s 60/379,927 provisional application, to which the patents-in-suit claim priority. See **Exhibit 3** chart comparing Quake to Ismagilov provisional application).

(iv) *Claim 6*

1831. Claim 6 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1832. Claim 6 further recites: “**the oil is fluorinated oil.**”

1833. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that

are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*”).

1834. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

1835. While it is my opinion that Quake discloses a fluorinated oil, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1836. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1837. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1838. It also would have been obvious to use a fluorinated oil based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

1839. Claim 7 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1840. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1841. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis

added).

1842. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel . . . The extrusion fluid may also contain one or more additives, *such as surfactants* . . .” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

1843. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating which minimizes adhesion . . . Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

1844. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the

device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(vi) *Claim 8*

1845. Claim 8 of the ’148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1846. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1847. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

1848. While it is my opinion that Quake discloses a fluorinated surfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1849. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may

also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1850. It also would have been obvious to use a fluorinated surfactant based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(b) Invalidity Based on Shaw Stewart

1851. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the ’148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1852. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

1853. Shaw Stewart satisfies this claim limitation. For example, Shaw Stewart described that a microfluidic system with at least two channels comprising at least one junction, as shown in Figure 1, reproduced below:

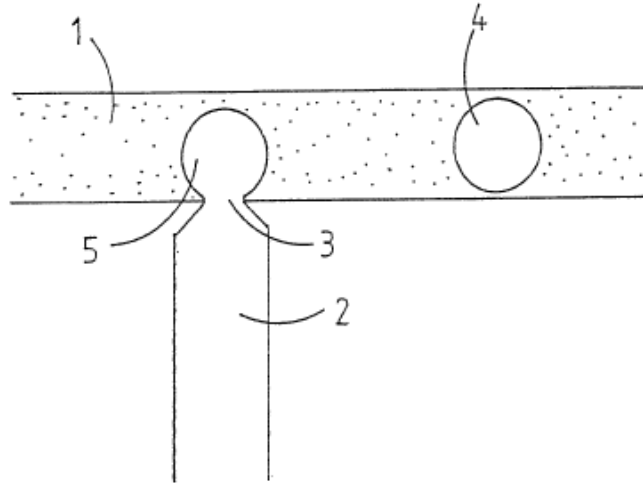


Figure 1.

Shaw Stewart at Fig. 1. In describing the figure, Shaw Stewart stated that “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1854. Figure 2 of Shaw Stewart, reproduced below, also discloses this limitation.

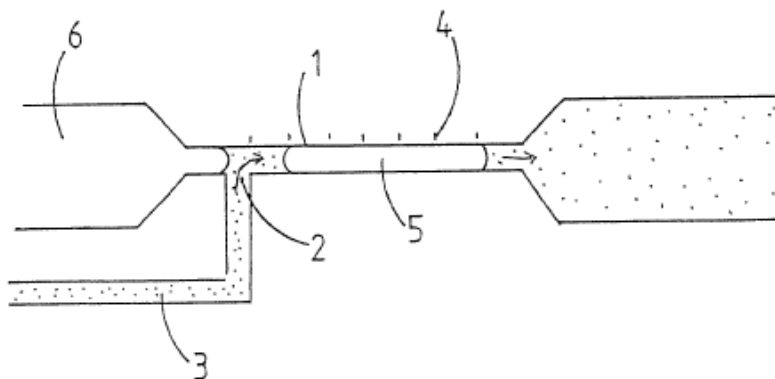


Figure 2.

Shaw Stewart at Fig. 2. In describing Figure 2, Shaw Stewart stated that “[i]n this case the reagent (6) is passed into a tube of considerably narrower bore (1) than the cross-section of the

droplets to be produced. The tube is graduated relative to the opening (2) of a side arm (3). The reagent is passed into the tube to a certain graduation (4), whereupon carrier phase is introduced from the side-arm, thus breaking off a droplet to the required size.” Shaw Stewart at 1:96-104.

1855. Claim 1 further recites: “**providing within the one or more channels a continuously carrier fluid comprising an oil.**”

1856. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

1857. Figure 1 of Shaw Stewart also discloses this limitation.

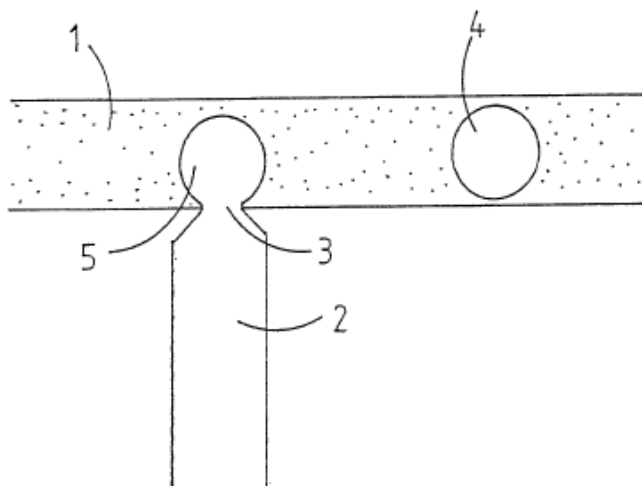


Figure 1.

1858. Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1859. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an

immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

1860. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

1861. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33. Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry,

biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added). Because double-stranded DNA is a highly stable molecule that does not react unless denatured (i.e., unwinds into single-stranded DNA), and denaturing of double-stranded DNA takes place in the presence of high temperatures or chemicals, a POSA would have understood that in the absence of high temperatures or chemicals, the double-stranded would not have reacted. Mullis at 9:55-60.

1862. Figure 1 of Shaw Stewart also discloses this limitation.

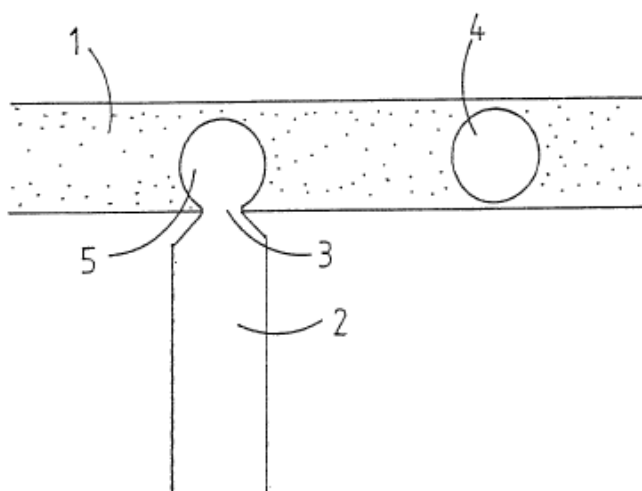


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

1863. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad

stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

1864. While it is my opinion that Shaw Stewart discloses a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1775-1777, demonstrating how Quake discloses this limitation.

1865. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow of reaction mixture were not reacting with each other.

1866. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that

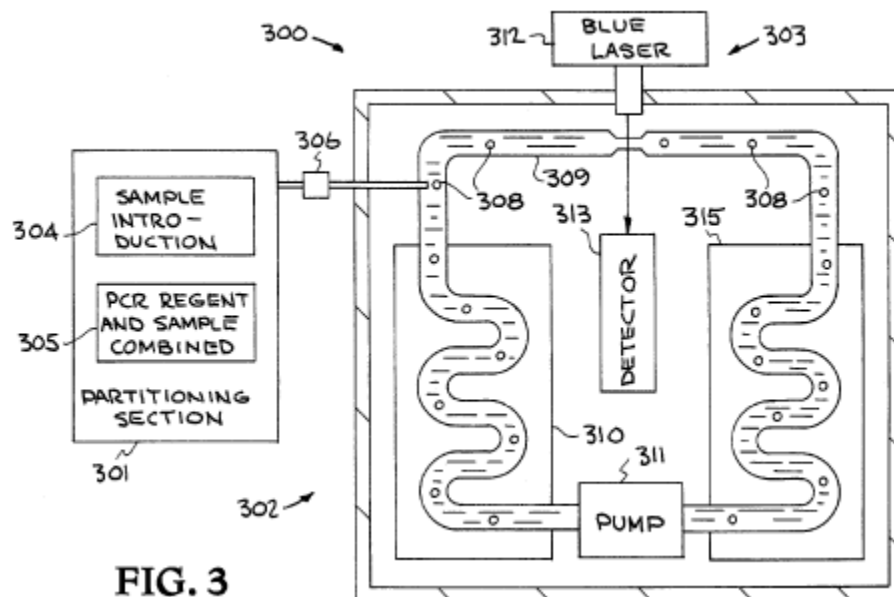
“[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1867. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1868. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes

relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

1869. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1870. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR

section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via

polymerase chain reaction (PCR).” Id. at 7:47-50.

1871. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1872. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.**”

1873. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

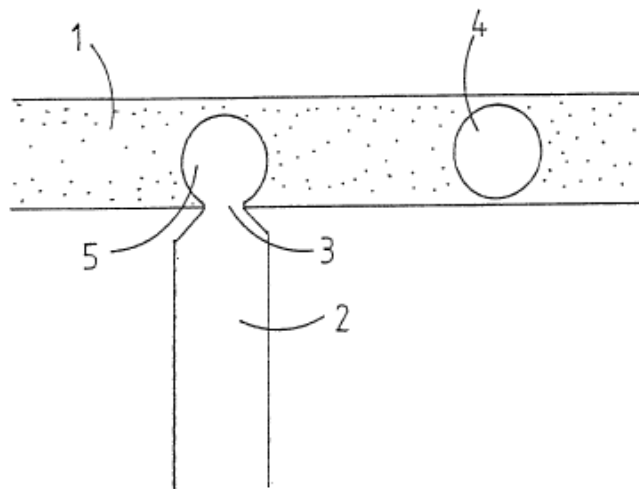


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small

opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75. Shaw Stewart thus describes “controlling” flow rates by “suck[ing] or push[ing]” the reagent stream through a small opening.

1874. Claim 1 further recites: **“each [plug] having a substantially uniform size of about 200µm or less.”**

1875. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1790-1791, demonstrating how Quake discloses that each plug has a substantially uniform size of about 200 µm or less.

1876. Claim 1 further recites: **“wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.”**

1877. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1793-1794, demonstrating how Quake discloses a Poisson distribution of target DNA or RNA in plugs.

1878. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product

peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

1879. It also would have been obvious to have a Poisson distribution of target DNA or RNA molecules based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

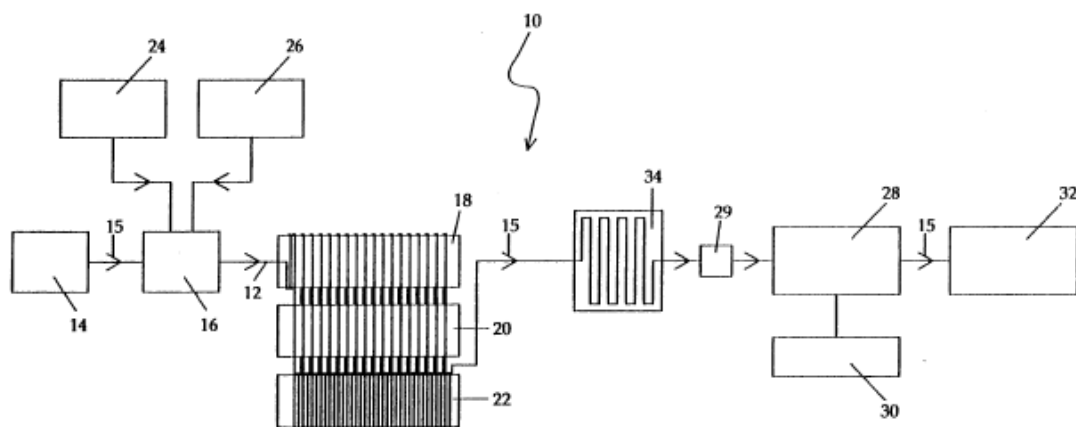
1880. Claim 1 further recites: **“and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule.”**

1881. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86.

1882. While it is my opinion that Shaw Stewart discloses a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at

6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1883. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire

microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1884. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA

would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1885. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nL” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1886. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

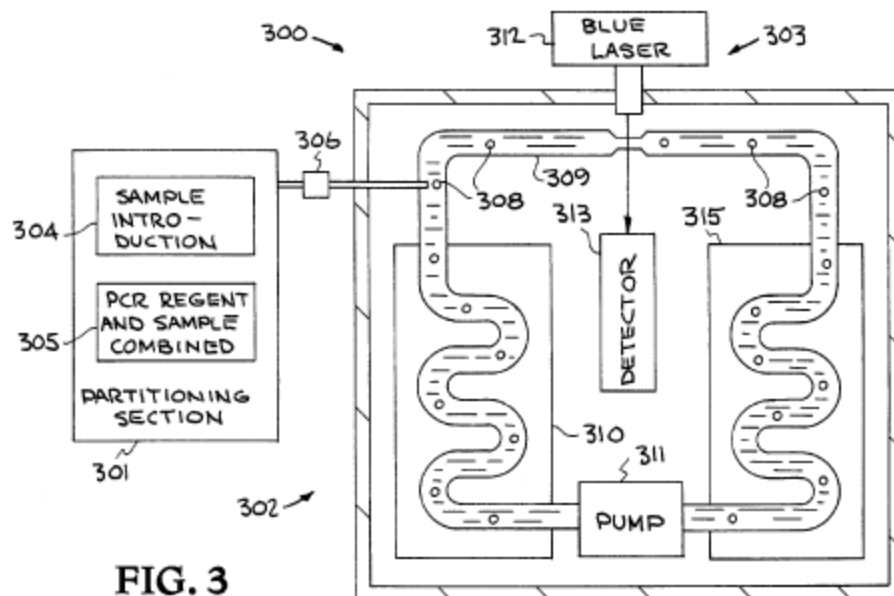


FIG. 3

1887. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:34-37.

1888. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

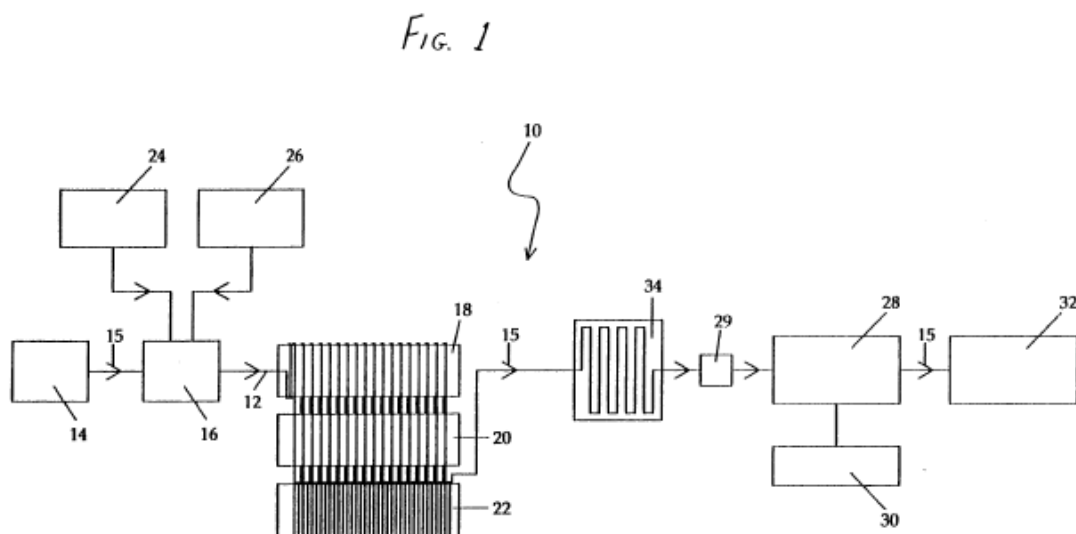
1889. Claim 1 further recites: “**and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.**”

1890. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would

allow different types of reactions to take place within droplets. Shaw Stewart described that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature*.” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

1891. While it is my opinion that Shaw Stewart discloses providing conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable

components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1892. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

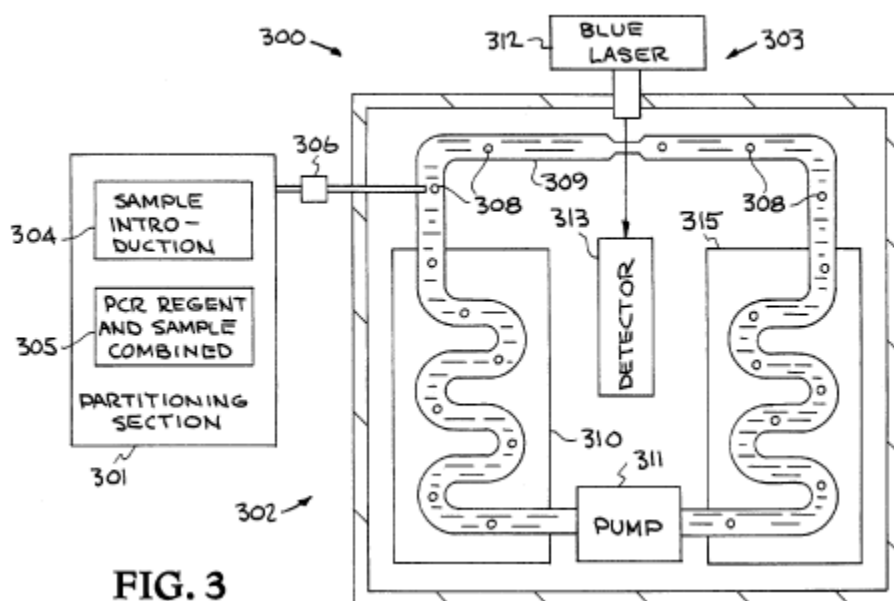
amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1893. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1894. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1

cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1895. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1896. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50.

1897. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and

Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1898. Claim 2 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1899. Claim 2 further recites: “**the step of providing conditions includes heating.**”

1900. Shaw Stewart satisfies this limitation. For example, Shaw Stewart disclosed providing a number of conditions, including heating and other temperature changes, that would allow different types of reactions to take place within droplets. Shaw Stewart described that “*[r]egions of the device can be heated* by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at *the required temperature.*” Shaw Stewart at 2:44-50 (emphasis added); *see also* Shaw Stewart at 3:70-72 (“More complex versions of the system using more reactants, and incubating the mixture at various temperatures are readily possible.”); Shaw Stewart at 3:57-60 (“If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.”).

1901. While it is my opinion that Shaw Stewart discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being

held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1902. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

1903. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1904. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within

droplets in a microfluidic system.

1905. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

1906. It also would have been obvious to provide heating to the microfluidic system based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

1907. Claim 3 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1908. Claim 3 further recites: “**providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.**”

1909. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Shaw Stewart at 2:59-64; *see also* Shaw Stewart at 2:64-66 (“For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.”). Shaw Stewart also described how reactions

resulting in color changes could be detected: “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil.” Shaw Stewart at 3:57-60.

1910. While it is my opinion that Shaw Stewart discloses providing a detector, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1826-1830, demonstrating how Quake discloses a detector.

1911. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1912. It also would have been obvious to provide a detector based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 6*

1913. Claim 6 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1914. Claim 6 further recites: “**the oil is fluorinated oil.**”

1915. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.” Shaw Stewart II at 4 (emphasis added).

1916. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1917. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1918. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1919. It also would have been obvious to use a fluorinated oil based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

1920. Claim 7 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1921. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

1922. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxyol, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains **a surfacting agent** which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(vi) *Claim 8*

1923. Claim 8 of the '148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

1924. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

1925. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it

would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1926. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1927. It also would have been obvious to use a fluorinated surfactant based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(c) Invalidity Based on Burns (2001)

1928. It is my opinion that the Burns (2001) discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the '148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

1929. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

1930. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel has been developed. The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a large enhancement in the interfacial mass transfer and the reaction rate. Mass transfer performance data has been obtained for a *glass chip-based reactor in a 380 μm wide channel* by monitoring the extraction of acetic acid from kerosene slugs as they moved along the reactor channel.” Burns (2001) at Abstract (emphasis added).

1931. Figure 4 from Burns (2001) also illustrates a microfluidic system with at least two channels having at least one junction:

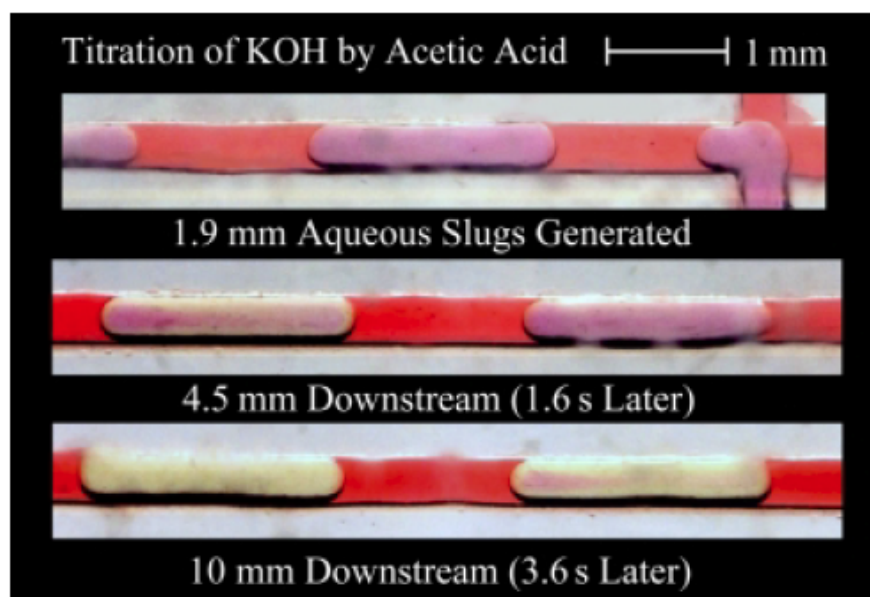


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1932. Claim 1 further recites: “**providing within the one or more channels a**

continuously carrier fluid comprising an oil.”

1933. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

1934. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1935. This reaction is illustrated in Figure 4:

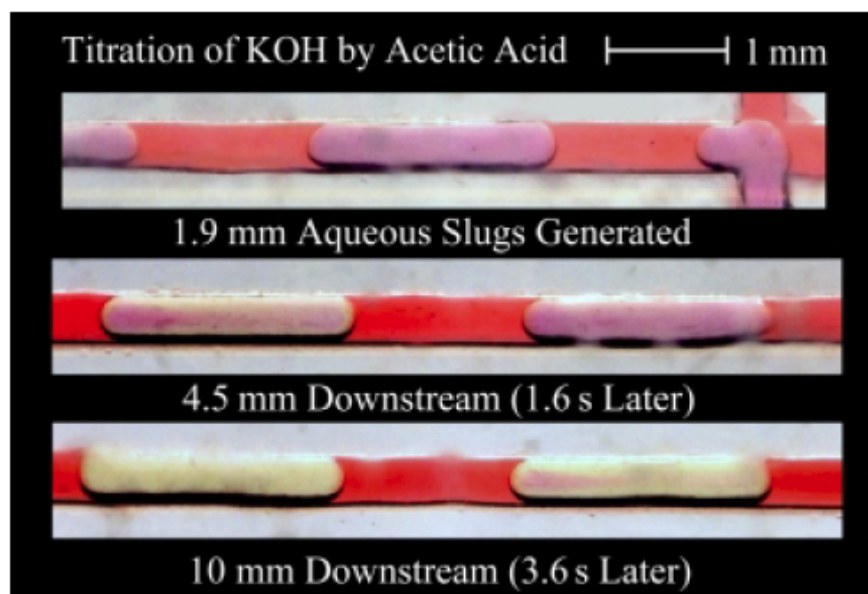


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1936. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

1937. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

1938. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

1939. This reaction is illustrated in Figure 4:

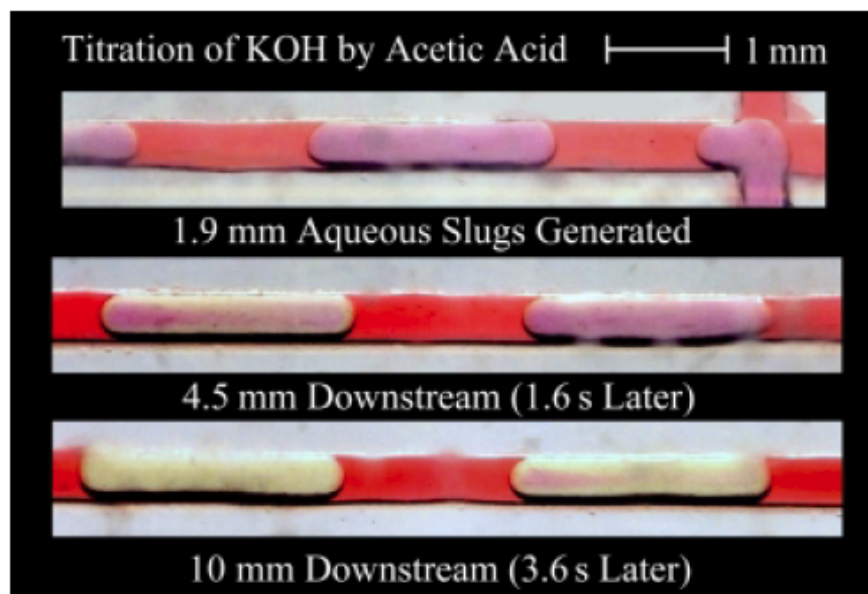


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1940. It would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1775-1777, demonstrating how Quake discloses this limitation.

1941. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow

of reaction mixture were not reacting with each other.

1942. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

1943. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions

pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1944. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

1945. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

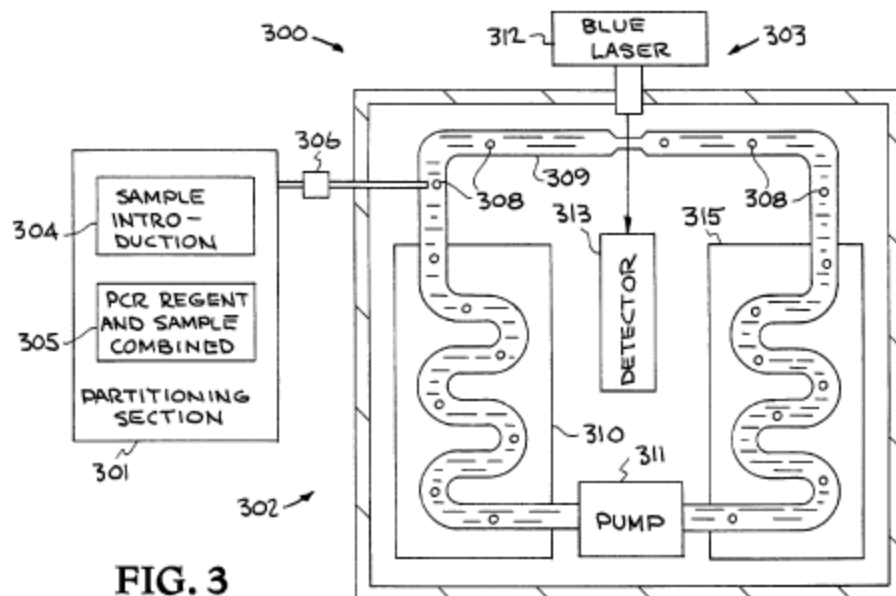


FIG. 3

1946. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Id. at 7:47-50.

1947. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1948. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.**”

1949. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped

intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added). Burns (2001) also describes that “[a] syringe driver loaded with two 1 ml glass syringes was attached to the tubing using a modified luer fitting and was used to control the flow to the device. The flow ratio was therefore fixed at 1:1 for all experiments.” Burns (2001) at 11.

1950. Figure 4 also illustrates this process:

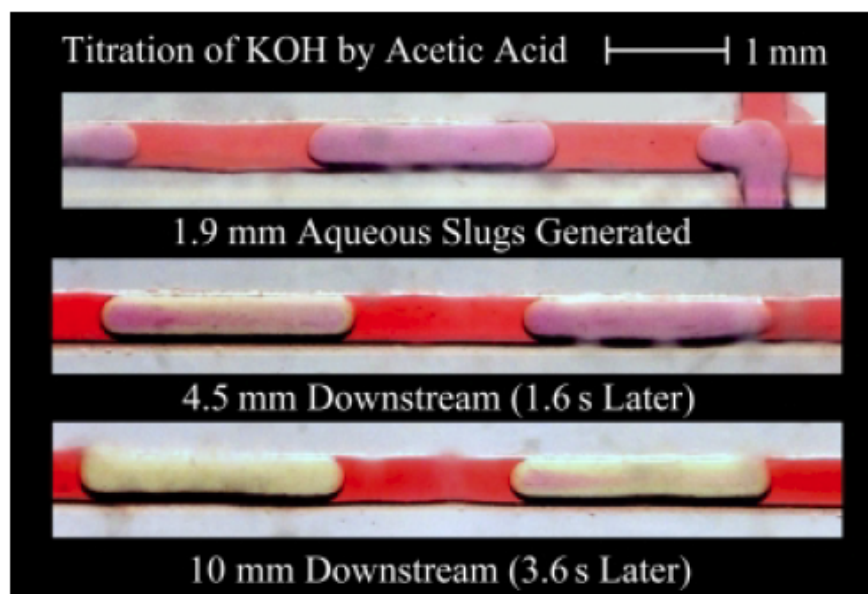


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1951. Claim 1 further recites: “each [plug] having a substantially uniform size of about 200 μ m or less.”

1952. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art

references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1790-1791, demonstrating how Quake discloses that each plug has a substantially uniform size of about 200 μm or less.

1953. It also would have been obvious for each plug to have a substantially uniform size of about 200 μm or less based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1954. Claim 1 further recites: “**wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.**”

1955. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1793-1794, demonstrating how Quake discloses a Poisson distribution of target DNA or RNA in plugs.

1956. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

1957. It also would have been obvious to have a Poisson distribution of target DNA or

RNA molecules based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1958. Claim 1 further recites: **“and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule.”**

1959. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid-base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

1960. This reaction is illustrated in Figure 4:

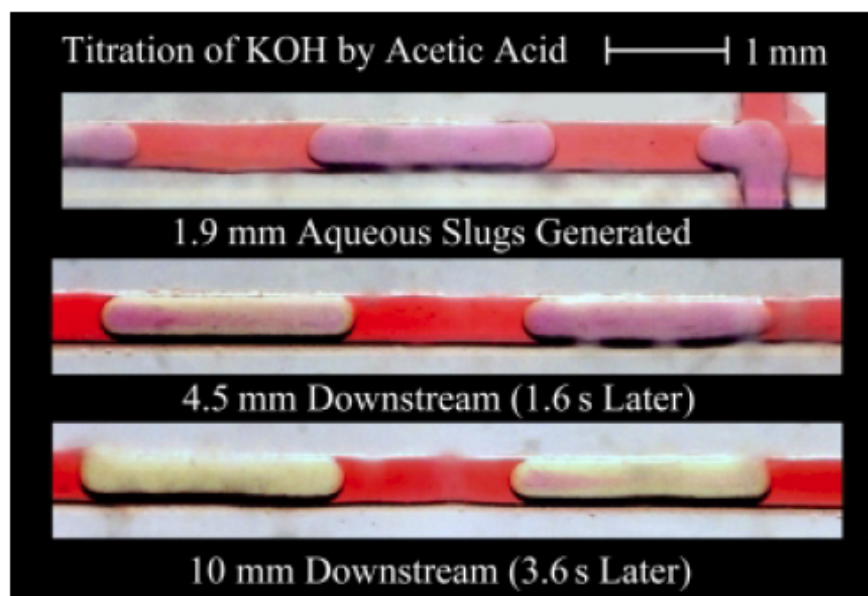
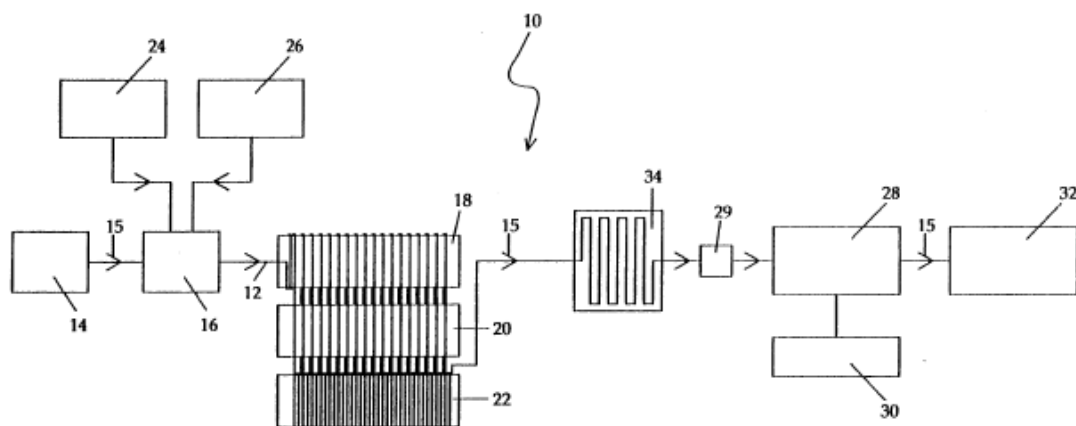


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1961. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1962. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

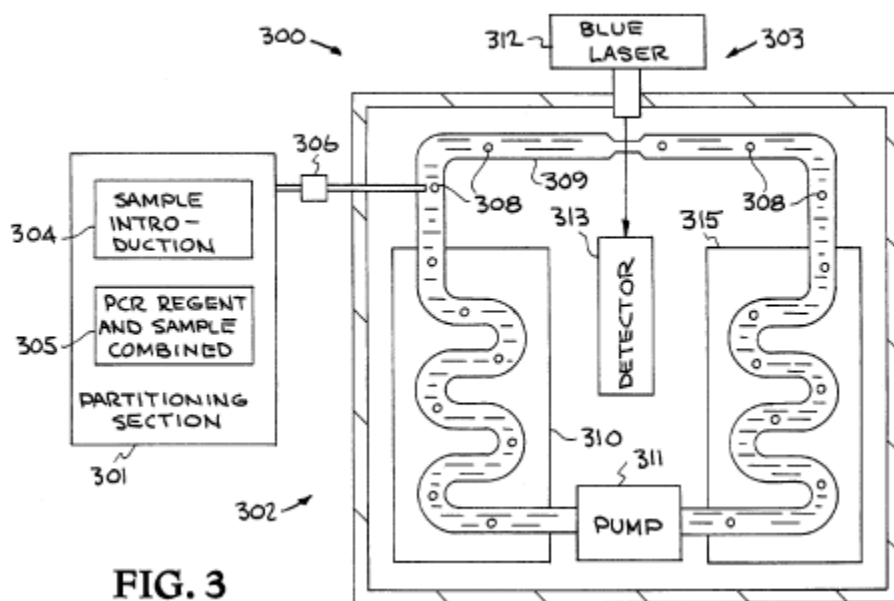
pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1963. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1964. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including

perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1965. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1966. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small

volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:35-36.

1967. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

1968. Claim 1 further recites: **“and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.”**

1969. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) provides conditions suitable for an acid-base reaction that occurs within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹

solutions.” Burns (2001) at 11.

1970. This reaction is illustrated in Figure 4:

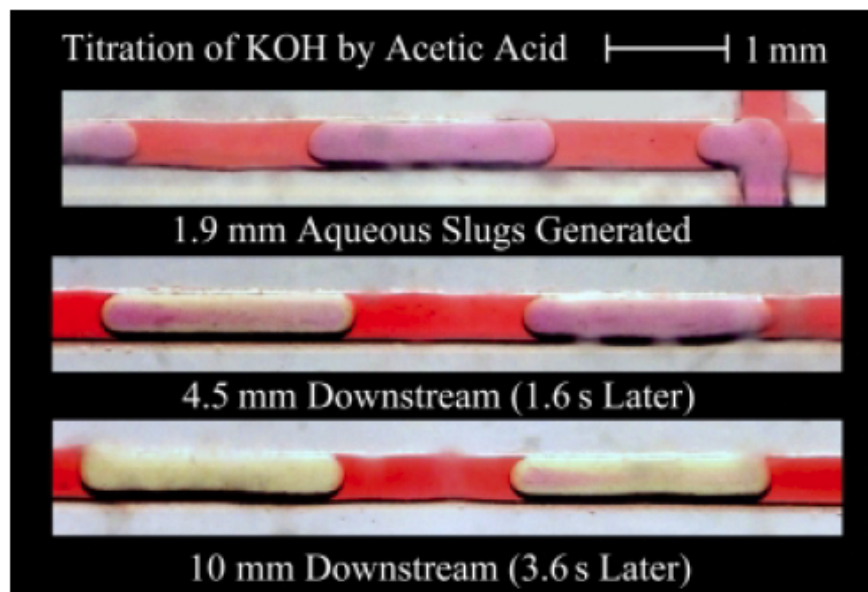


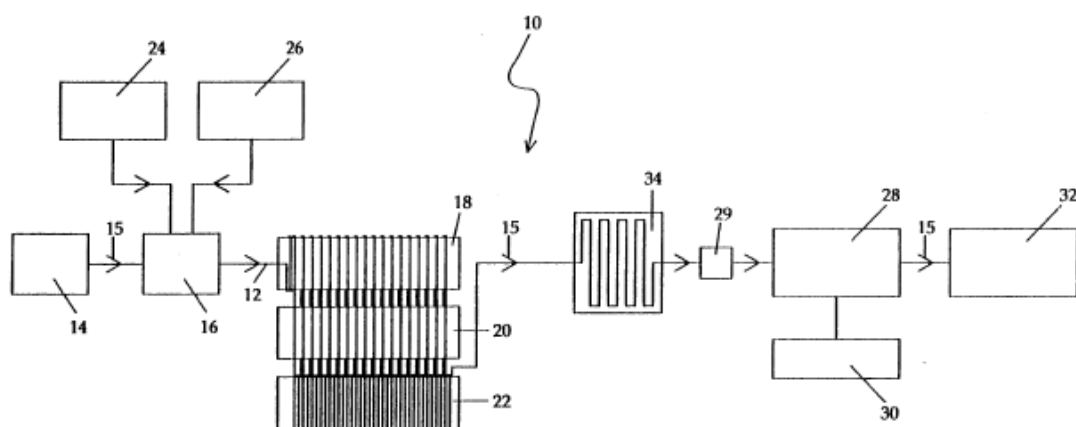
Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

1971. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample

containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1972. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a

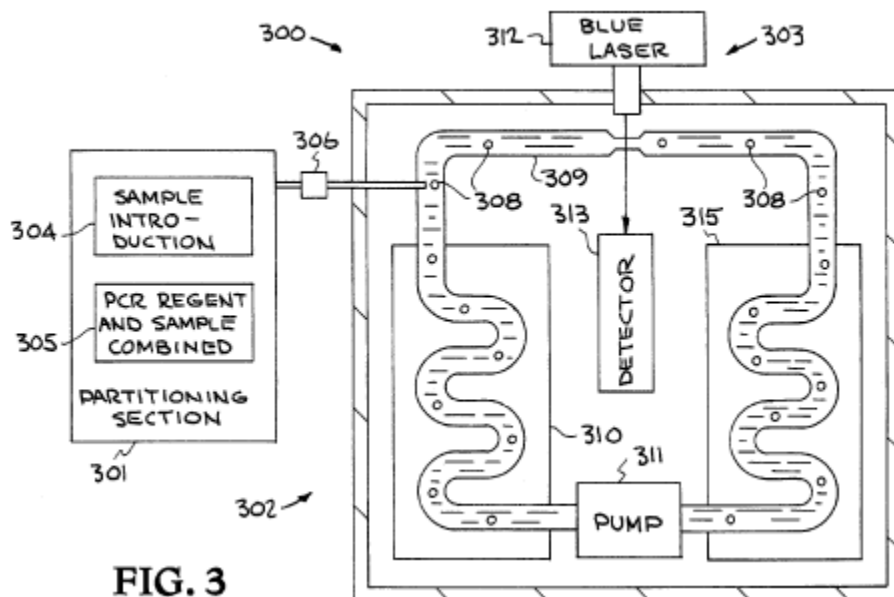
syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1973. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1974. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes

relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

1975. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



1976. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR

section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via

polymerase chain reaction (PCR).” Anderson at 7:47-50.

1977. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

1978. Claim 2 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1979. Claim 2 further recites: “**the step of providing conditions includes heating.**”

1980. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

1981. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL

chamber.” Lagally at Fig. 1.

1982. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

1983. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5.

1984. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such

as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

1985. It also would have been obvious to provide heating to the microfluidic system based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

1986. Claim 3 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1987. Claim 3 further recites: “**providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.**”

1988. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses that “[p]hotographs were taken of the flow using a 35 mm camera with a reversed 24 mm macro lens and up to 55 mm extension tubes. A flash gun was targeted at a white board mounted 6 cm below the chip to provide back illumination. Colour change was observed within the aqueous slug as acetic acid diffused into the aqueous phase reacting with the KOH or NaOH and changing the pH of the slug.” Burns (2001) at 11.

1989. While it is my opinion that Burns (2001) discloses providing a detector, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1826-1830, demonstrating how Quake discloses a detector.

1990. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n

a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

1991. It also would have been obvious to provide a detector based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 6*

1992. Claim 6 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

1993. Claim 6 further recites: “**the oil is fluorinated oil.**”

1994. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) discloses that “[*k*]erosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation.” Burns (2001) at 11 (emphasis added).

1995. It would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

1996. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft

describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

1997. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

1998. It also would have been obvious to use a fluorinated oil based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

1999. Claim 7 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2000. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

2001. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it

would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2002. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2003. It also would have been obvious to use a surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

2004. Claim 8 of the ’148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

2005. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

2006. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated

surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2007. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2008. It also would have been obvious to use a fluorinated surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(d) Invalidity Based on Nisisako

2009. It is my opinion that the Nisisako discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the '148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

2010. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

2011. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil

as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep.*” Nisisako at Abstract (emphasis added).

2012. The figures in Nisisako also disclose this limitation. For example, Figures 1 and 2 both show a microfluidic system with two channels meeting at a junction:

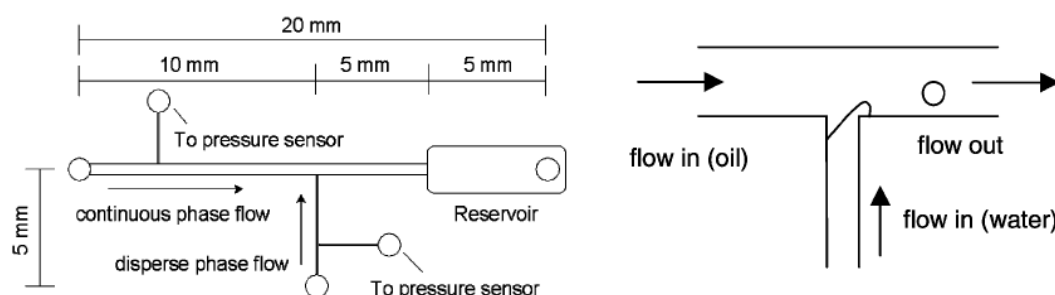


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

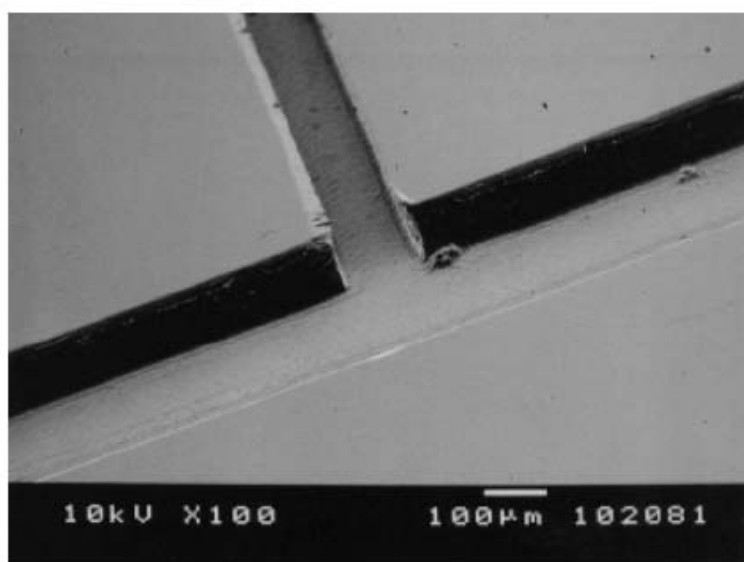


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

2013. Claim 1 further recites: “**providing within the one or more channels a**

continuously carrier fluid comprising an oil.”

2014. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and **high oleic sunflower oil (triolein, 80%) as the continuous phase**. Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that **the flow rate is constant.**”).

2015. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

2016. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, . For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, **pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.**” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. **Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps.** No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-

5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that *the flow rate is constant.*”).

2017. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

2018. It would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1775-1777, demonstrating how Quake discloses this limitation.

2019. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA

that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow of reaction mixture were not reacting with each other.

2020. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

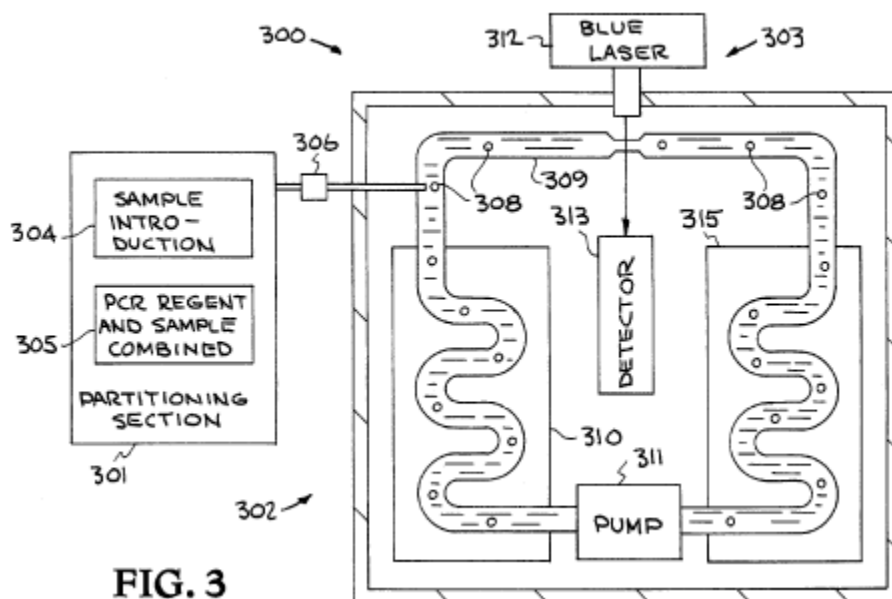
2021. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the

target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2022. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

2023. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.”

Anderson at Abstract. Figure 3 of Anderson is reproduced below:



2024. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **309** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Id. at 7:47-50.

2025. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2026. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.**”

2027. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil

as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps. No surfactant is added to either phase. *Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan)* are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating water-in-oil droplets in a microchannel network.”).

2028. Nisisako also discloses that “[t]his method of droplet formation is shown schematically in Fig. 1.” Nisisako at 24. Figure 1 is reproduced below:

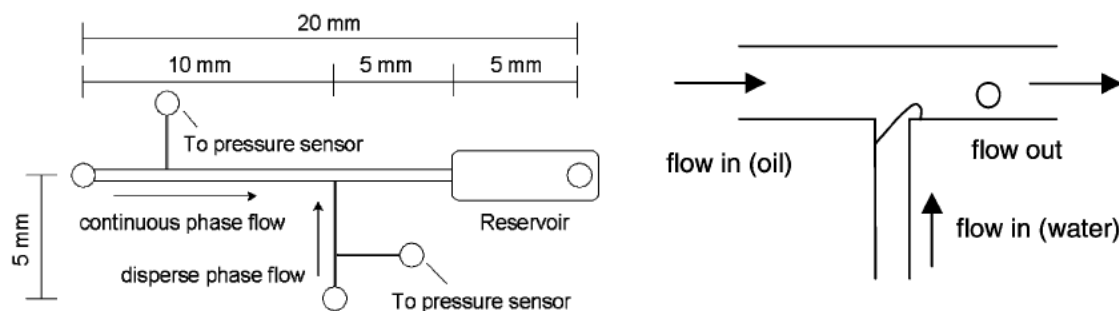


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

Nisisako at Fig. 1. Droplet formation at the T-junction is also illustrated by Figure 3:

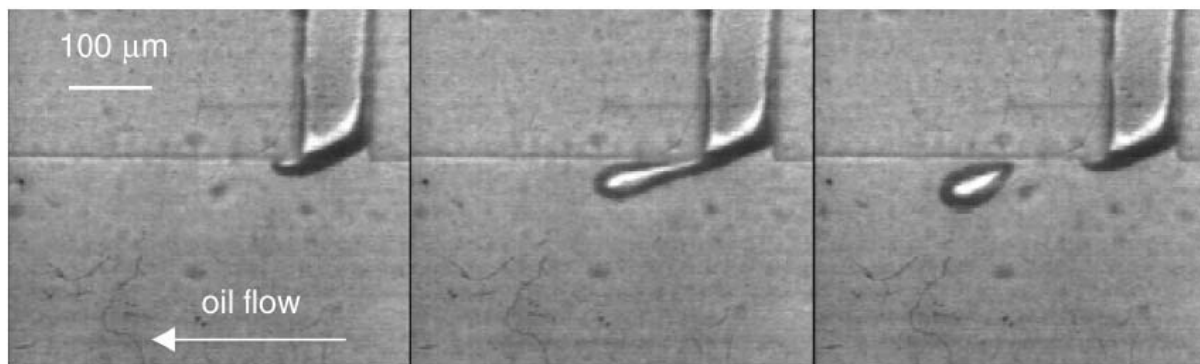


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Fig. 3; *see also* Nisisako at 25 (“Regular-sized droplets of water in oil were generated at the T-junction (Fig. 3).”

2029. Claim 1 further recites: “each [plug] having a substantially uniform size of about 200μm or less.”

2030. Nisisako satisfies this limitation. For example, Nisisako describes that “[t]he droplet size can be controlled: the minimum diameter of the droplets was about 100 μm, and the maximum 380 μm, as the flow velocity of the continuous phase was changed from 0.01 to 0.15 m s⁻¹.” Nisisako at 26. In Figure 6, Nisisako plots volume against velocity, and shows that all velocities tested, the volume of a droplet was below 200 μm:

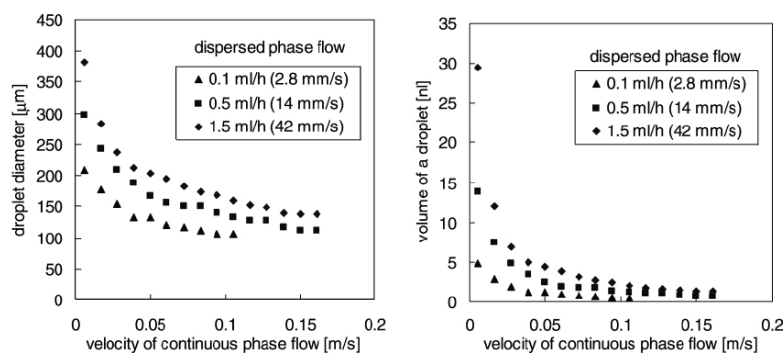


Fig. 6 Effect of velocity of continuous phase flow on droplet size (left: droplet diameter data, right: volume of a droplet calculated from the diameter).

Nisisako at Fig. 6.

2031. Nisisako also makes clear that the droplets are substantially uniform in size:

“Regular-sized droplets of water in oil were generated at the T-junction.” Nisisako at 25 (emphasis added).

2032. Claim 1 further recites: **“wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.”**

2033. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1793-1794, demonstrating how Quake discloses a Poisson distribution of target DNA or RNA in plugs.

2034. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

2035. It also would have been obvious to have a Poisson distribution of target DNA or RNA molecules based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

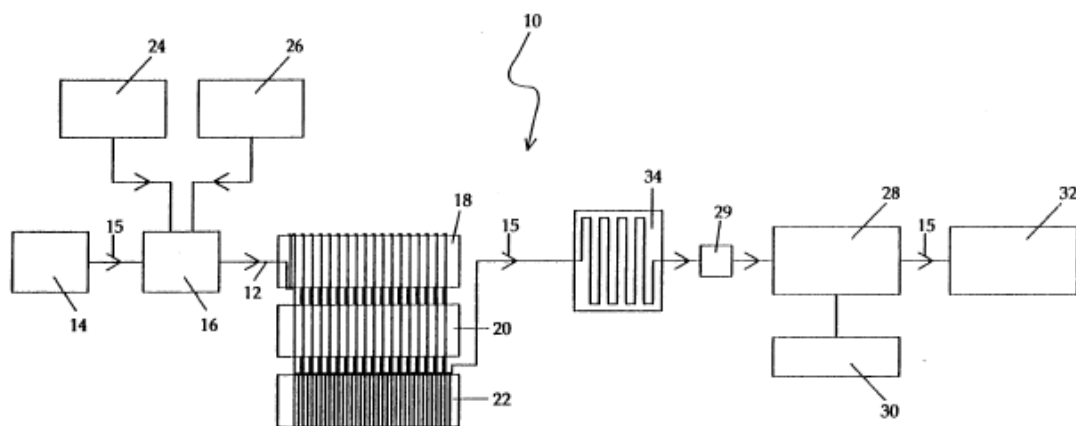
2036. Claim 1 further recites: **“and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react**

with the target DNA or RNA molecule.”

2037. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako makes clear that the droplets described can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

2038. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2039. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

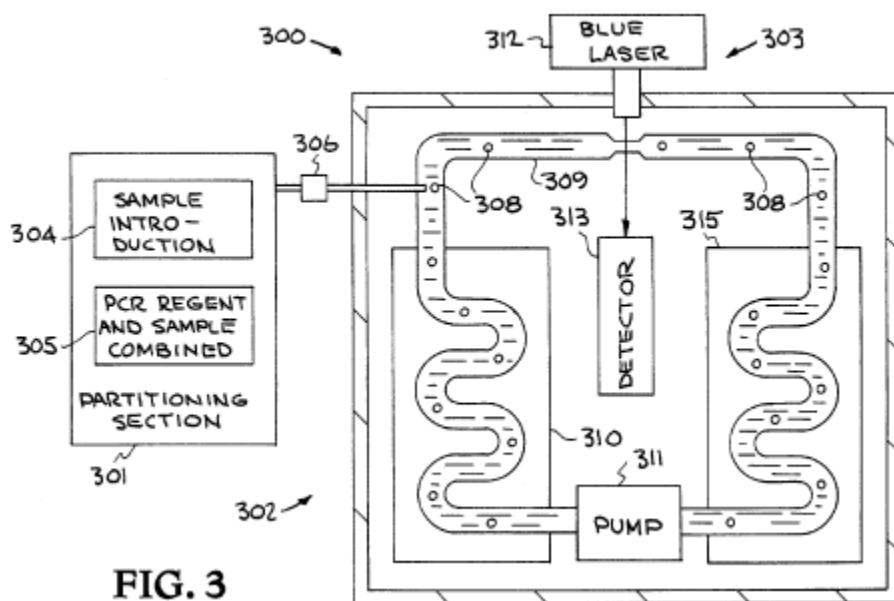
pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2040. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2041. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including

perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2042. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



2043. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small

volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:34-37.

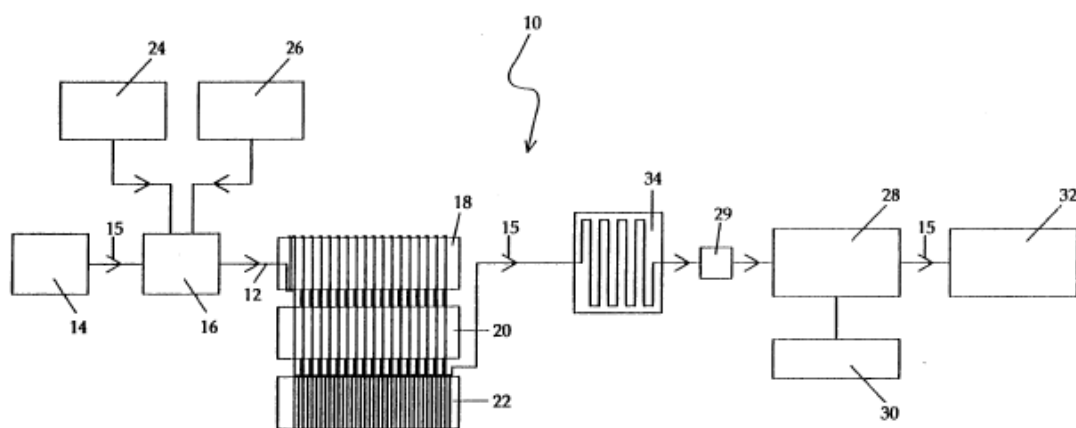
2044. It also would have been form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2045. Claim 1 further recites: “**and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.**”

2046. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the

DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2047. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1

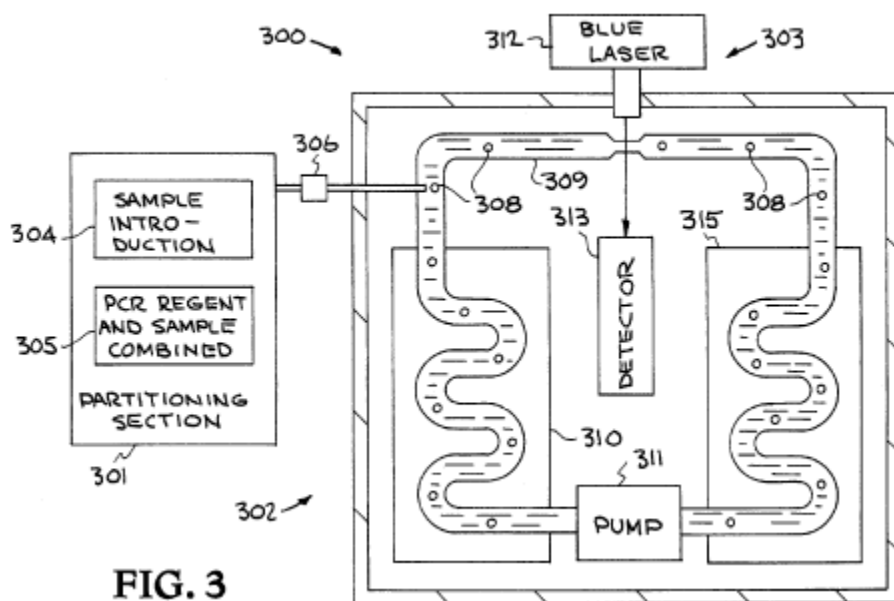
x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2048. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2049. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible

organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nL” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2050. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



2051. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50.

2052. It also would have been provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

2053. Claim 2 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2054. Claim 2 further recites: **“the step of providing conditions includes heating.”**

2055. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

2056. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

2057. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2058. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5.

2059. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

2060. It also would have been obvious to provide heating to the microfluidic system based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

2061. Claim 3 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2062. Claim 3 further recites: **“providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.”**

2063. Nisisako satisfies this limitation. For example, Nisisako describes that “[d]roplet formation at the T-junction is observed using a microscope (BX50; Olympus, Japan) and a high-speed video camera (FASTCAM-ultima; Photron, Japan).” Nisisako at 25.

2064. While it is my opinion that Nisisako discloses providing a detector, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1826-1830, demonstrating how Quake discloses a detector.

2065. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

2066. It also would have been obvious to provide a detector based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 6*

2067. Claim 6 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2068. Claim 6 further recites: **“the oil is fluorinated oil.”**

2069. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2070. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2071. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or

manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2072. It also would have been obvious to use a fluorinated oil based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

2073. Claim 7 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2074. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

2075. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2076. It also would have been obvious to use a surfactant in view of Krafft. Krafft

describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2077. It also would have been obvious to use a surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

2078. Claim 8 of the '148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

2079. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

2080. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2081. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may

also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2082. It also would have been obvious to use a fluorinated surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(e) Invalidity Based on Thorsen

2083. It is my opinion that the Thorsen discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the '148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

2084. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

2085. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at *the junction of two microfluidic channels* containing water and an oil mixture, respectively.” Thorsen at 4163 (emphasis added); *see also* Thorsen at Abstract (“Here, we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

2086. Thorsen also describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring acrylated urethane (Ebecryl 270, UCB Chemicals) on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned

urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x 6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. Figure 1, showing a microfluidic system with two channels and a junction, is shown below:

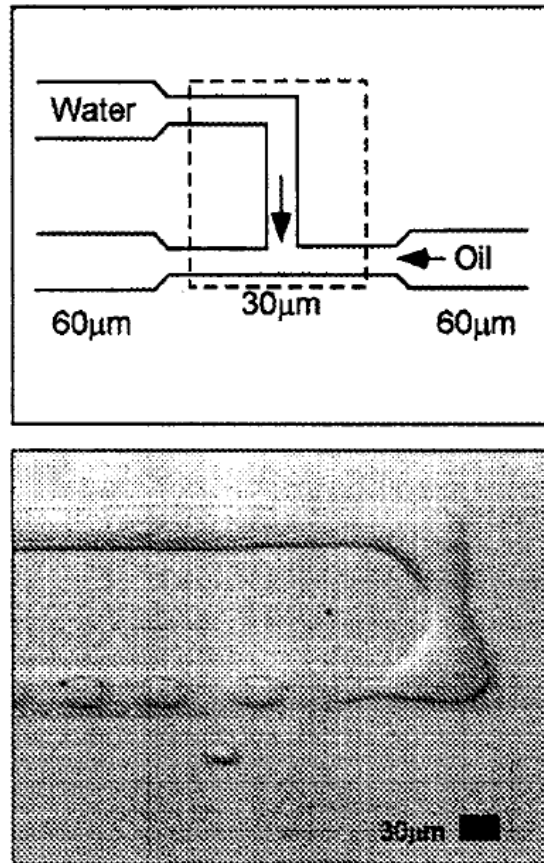


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

2087. Claim 1 further recites: “**providing within the one or more channels a continuously carrier fluid comprising an oil.**”

2088. Thorsen satisfies this limitation. For example, Thorsen describes that

“[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

2089. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

2090. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

2091. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, . . . For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

2092. Thorsen also discloses that “[t]he fluids are introduced into the urethane

microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164.

“As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

2093. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to DNA or RNA.

2094. It would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1775-1777, demonstrating how Quake discloses this limitation.

2095. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for

which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow of reaction mixture were not reacting with each other.

2096. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was

introduced at one of the sample bus reservoirs with a pipet.” Lagally at 567.

2097. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2098. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

2099. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and

the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.”

Anderson at Abstract. Figure 3 of Anderson is reproduced below:

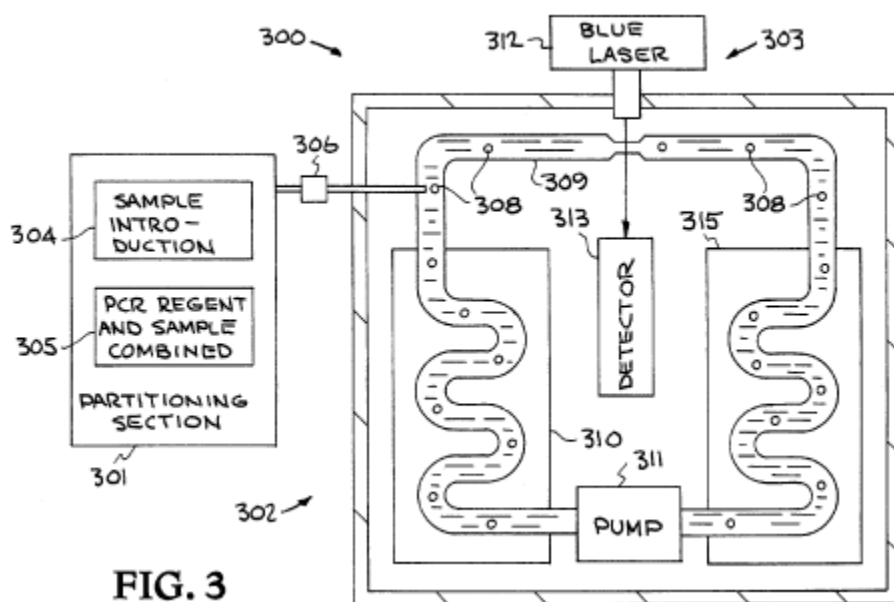


FIG. 3

2100. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Id. at 7:47-50.

2101. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2102. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said**

carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.”

2103. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

2104. Thorsen also discloses that the flow rates of the aqueous fluid and the oil are controlled, describing that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the *relative water pressure is increased at fixed oil pressure*, the droplets become ordered into a single continuous stream.” Thorsen at 4163 (emphasis added).

2105. Figure 1, showing plug formation, is reproduced below:

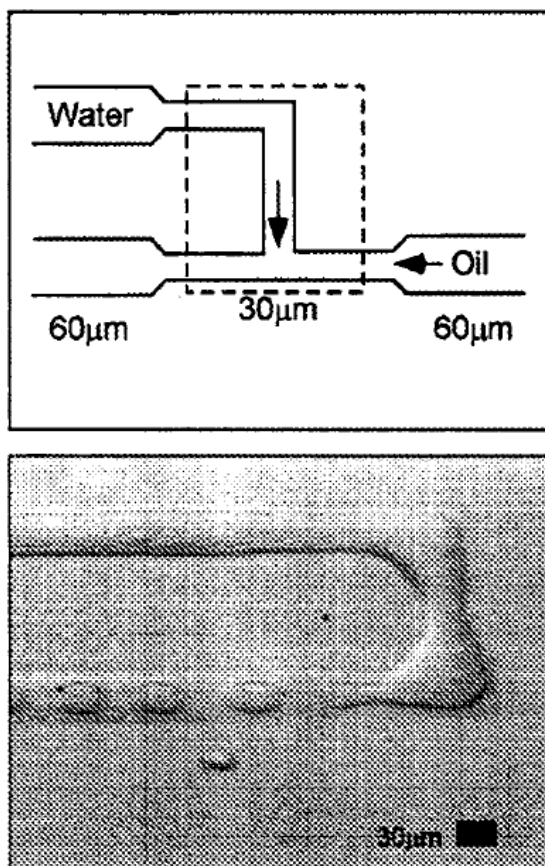


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

2106. Claim 1 further recites: “each [plug] having a substantially uniform size of about 200µm or less.”

2107. Thorsen satisfies this limitation. For example, Thorsen describes that “[d]roplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating *picoliter-scale* droplets.” Thorsen at 4163 (emphasis added).

2108. Thorsen also describes that the plugs created have substantially uniform size. For example, Thorsen states that “[u]nder conditions where the water pressure is lower than the oil

pressure, *monodisperse separated reverse micelles are formed.*” Thorsen at 4163 (emphasis added); *see also* Thorsen at 2164 (“As the water pressure is increased past a critical point, single monodisperse separated droplets are formed at a frequency of 20-80 Hz.”).

2109. Claim 1 further recites: “**wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.**”

2110. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1793-1794, demonstrating how Quake discloses a Poisson distribution of target DNA or RNA in plugs.

2111. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

2112. It also would have been obvious have a Poisson distribution of target DNA or RNA molecules based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2113. Claim 1 further recites: “**and at least one member of said plurality comprises a**

single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule.”

2114. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

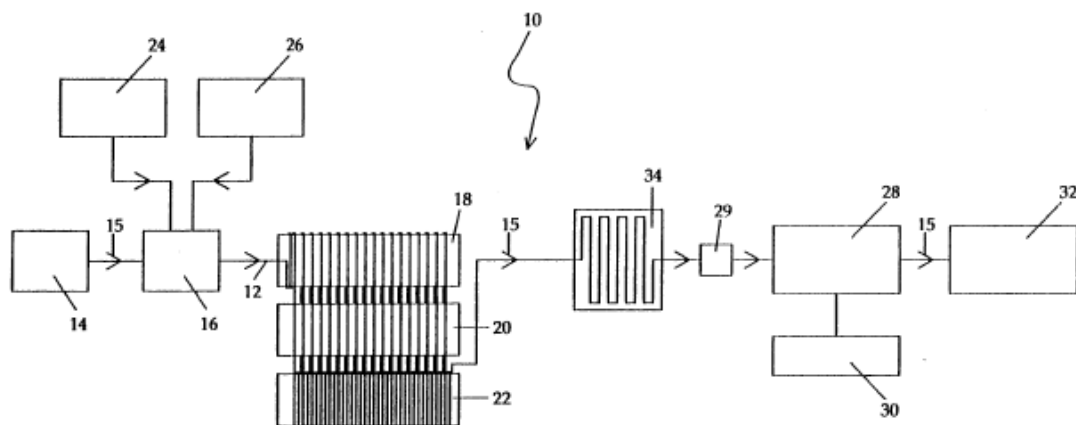
2115. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

2116. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to DNA or RNA.

2117. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the

[polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2118. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single

DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2119. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the

device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2120. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2121. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

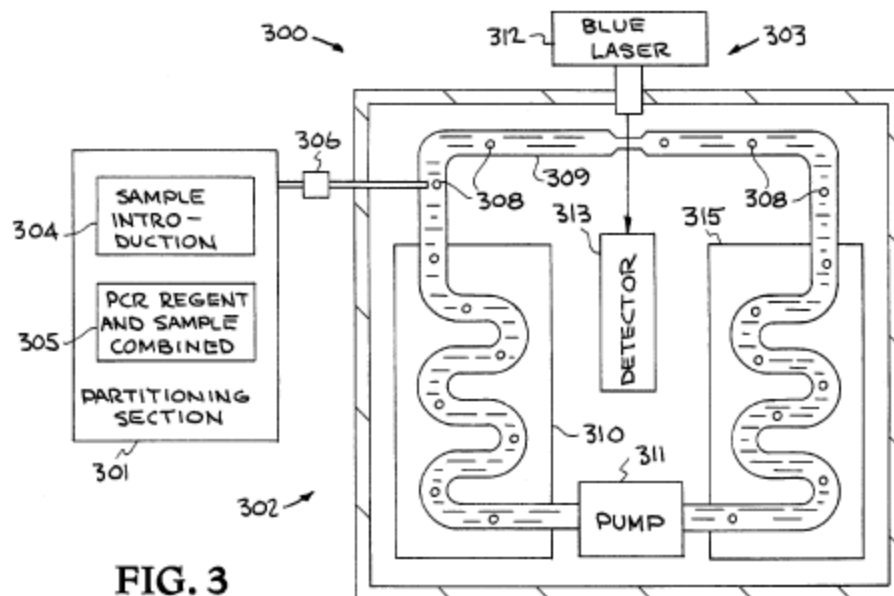


FIG. 3

2122. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:34-37.

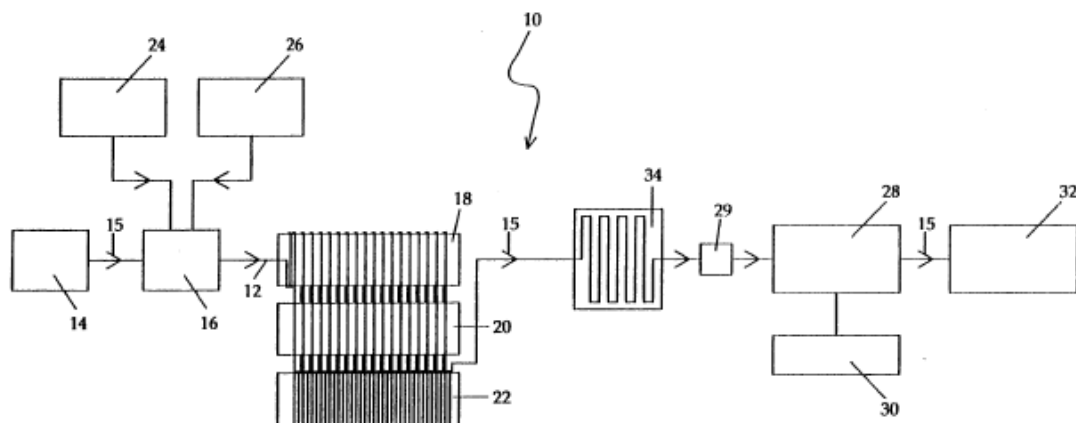
2123. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2124. Claim 1 further recites: “**and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.**”

2125. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it

would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2126. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2127. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns

(1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2128. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2129. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

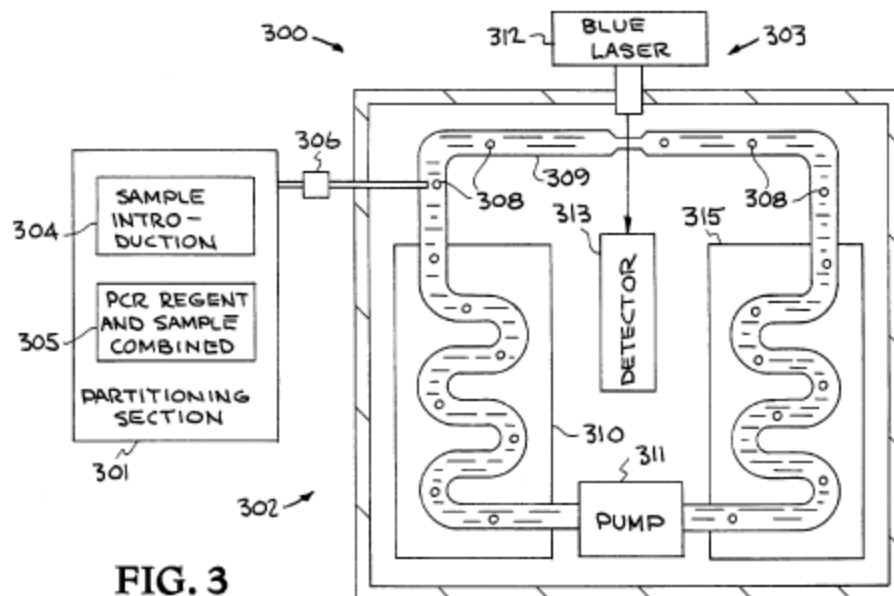


FIG. 3

2130. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50.

2131. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

2132. Claim 2 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2133. Claim 2 further recites: “**the step of providing conditions includes heating.**”

2134. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett discloses that “the present invention

relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

2135. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

2136. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2137. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise:

genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5.

2138. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

2139. It also would have been obvious to provide heating to the microfluidic system based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

2140. Claim 3 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2141. Claim 3 further recites: “**providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.**”

2142. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it

also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1826-1830, demonstrating how Quake discloses a detector.

2143. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

2144. It also would have been obvious to provide a detector based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 6*

2145. Claim 6 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2146. Claim 6 further recites: “**the oil is fluorinated oil.**”

2147. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential

for biomedical applications” and “are chemically and biologically stable.” *Id.*

2148. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2149. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2150. It also would have been obvious to use a fluorinated oil based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

2151. Claim 7 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2152. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

2153. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

2154. Thorsen further describes that “[v]arious oils were tested in the device, including decane, tetradecane, and hexadecane, *combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.*” Thorsen at 4164 (emphasis added).

(vi) Claim 8

2155. Claim 8 of the ’148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

2156. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

2157. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

2158. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2159. It also would have been obvious to use a fluorinated surfactant based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to

Combine and Reasonable Expectation of Success), and all references cited therein.

(f) Invalidity Based on Seki

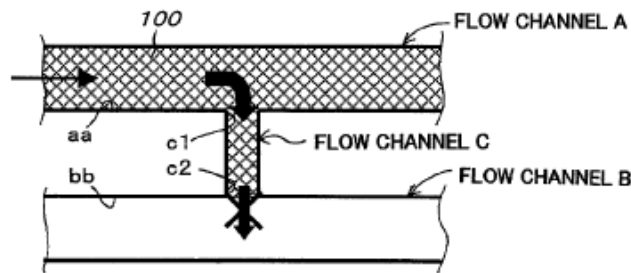
2160. It is my opinion that the Seki discloses and/or renders obvious all elements of claims 1-3 and 6-8 of the '148 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

2161. Claim 1 recites: “**A method comprising the steps of: providing a microfluidic system comprising one or more channels.**”

2162. Seki satisfies this limitation. For example, Seki describes a microfluidic system with at least two junctions having at least one junction:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.



Seki at Abstract.

2163. Claim 1 further recites: **“providing within the one or more channels a continuously carrier fluid comprising an oil.”**

2164. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system in which a carrier fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

2165. It also would have been obvious to use an oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary

fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2166. It also would have been obvious to use an oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2167. It also would have been obvious to use an oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

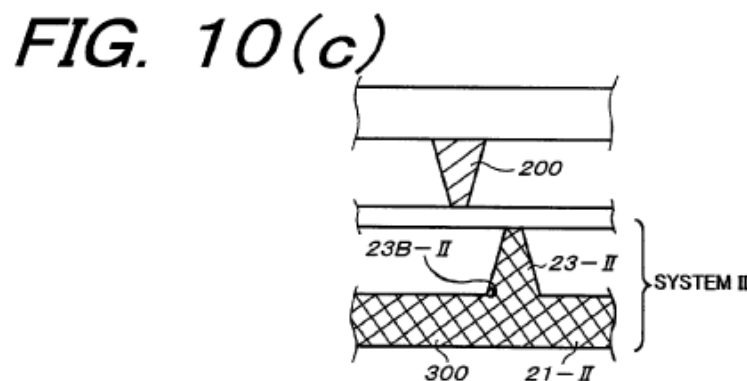
2168. Claim 1 further recites: “**and a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other.**”

2169. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

2170. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

2171. It would have been obvious to combine the teachings of Seki with one or more

prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1775-1777, demonstrating how Quake discloses this limitation.

2172. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes that

the present invention consists in a device for use in amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction, the device comprising a tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), suitable oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components into the carrier fluid; pump means adapted to maintain the flow of the carrier fluid through the tube; a plurality of zones at differing temperatures into contact with which the tube containing the stream of carrier fluid is brought, the differing temperatures and the time for which the carrier fluid stream containing the reaction mixture is in contact with the individual zones being selected such that the following reactions take place in the reaction mixture: (a) denaturation of the DNA strands in the sample, (b) annealing of the oligonucleotide primers with complementary sequences in the sample DNA, and (c) primed synthesis of new strands of complementary DNA that each extend beyond the site of annealing of the alternate primer; and recovery means adapted to allow removal of the reaction mixture from the carrier fluid following amplification of the specific target DNA sequence(s) present in the sample.

Corbett at 4:24-52. Because “denatur[ing] of the DNA strands in the sample” takes place in the presence of heat, a POSA would have understood that DNA molecules and reagents in the flow of reaction mixture were not reacting with each other.

2173. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2174. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Burns (1996). Burns

(1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2175. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. *Id.* at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” *Id.* at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.*

2176. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

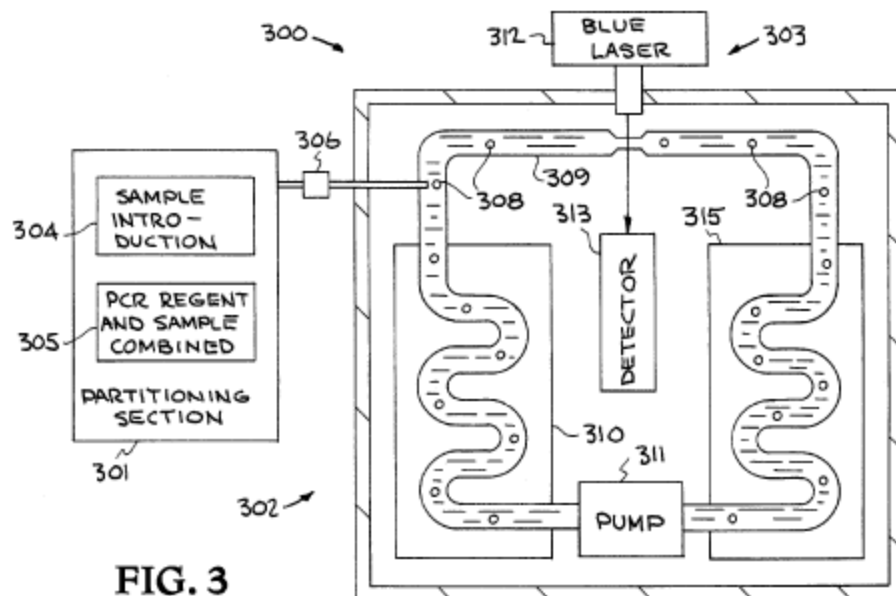


FIG. 3

2177. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Id. at 7:47-50.

2178. It also would have been obvious to continuously flow aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecule under conditions in which the target DNA or RNA molecule and the other molecules in the fluid do not react with each other based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2179. Claim 1 further recites: “**controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid.**”

2180. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow

channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

2181. Claim 1 further recites: **“each [plug] having a substantially uniform size of about 200µm or less.”**

2182. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025].

2183. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1790-1791, demonstrating how Quake discloses that each plug has a substantially uniform size of about 200 µm or less.

2184. It also would have been obvious for each plug to have a substantially uniform size of about 200 µm or less based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all

references cited therein.

2185. Claim 1 further recites: “**wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution.**”

2186. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1793-1794, demonstrating how Quake discloses a Poisson distribution of target DNA or RNA in plugs.

2187. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Lagally describes that “[r]epetitive PCR analyses at the single DNA template molecule level exhibit quantized product peak areas; a histogram of the normalized peak areas reveals clusters of events caused by 0, 1, 2, and 3 viable template copies in the reactor and these event clusters are shown to fit a Poisson distribution.” Lagally at Abstract.

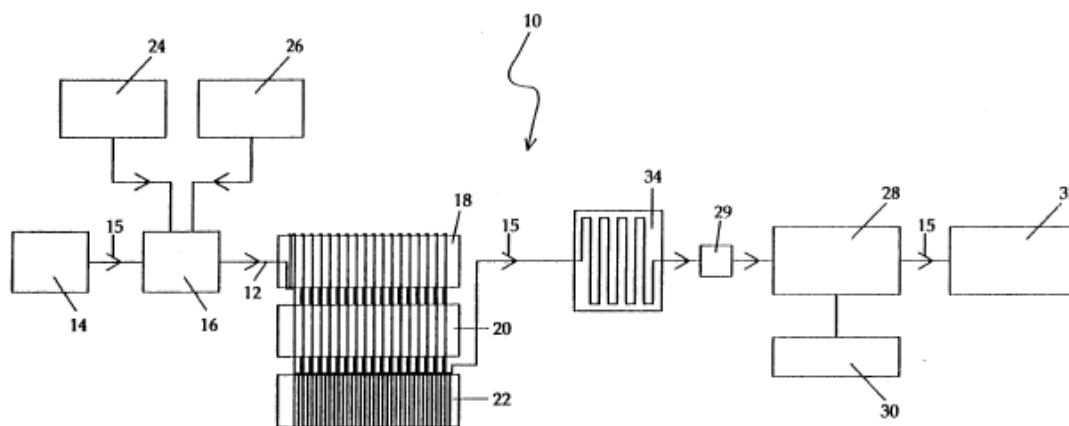
2188. It also would have been obvious to have a Poisson distribution of target DNA or RNA molecules based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2189. Claim 1 further recites: “**and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule.**”

2190. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes droplets comprising at least one biological molecule and at least one reagent for conducting the reaction with the at least biological molecule. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

2191. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” *Id.* at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2192. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

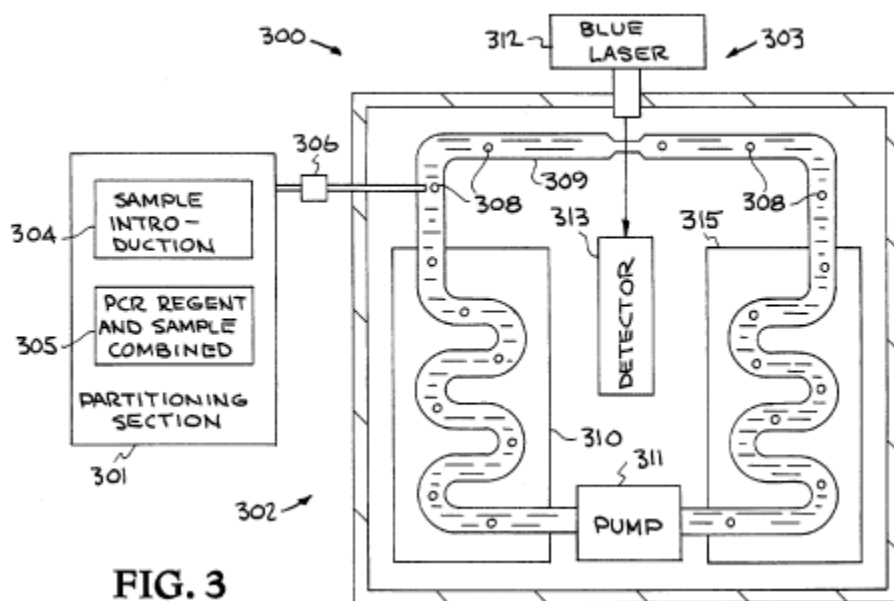
pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Further, Lagally describes that “we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates.” Lagally at 566. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2193. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2194. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including

perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2195. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:



2196. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50. Further, “given the extremely small

volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” Anderson at 7:34-37.

2197. It also would have been obvious to form a plug comprising a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

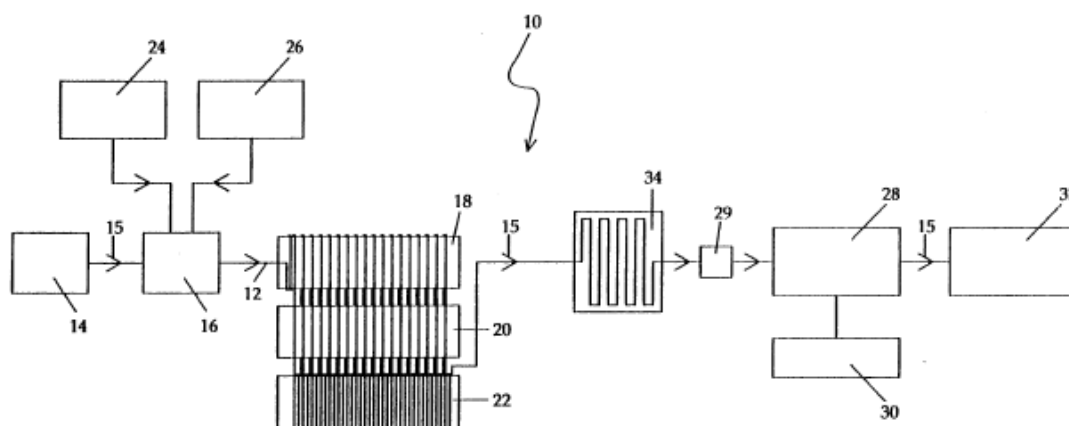
2198. Claim 1 further recites: “**and providing conditions suitable for a polymerase-chain reaction in the at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.**”

2199. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes the conditions suitable for conducting a glucose reaction: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

2200. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2201. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally

discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2202. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based

on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2203. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaq polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” *Id.* Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2204. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. Figure 3 of Anderson is reproduced below:

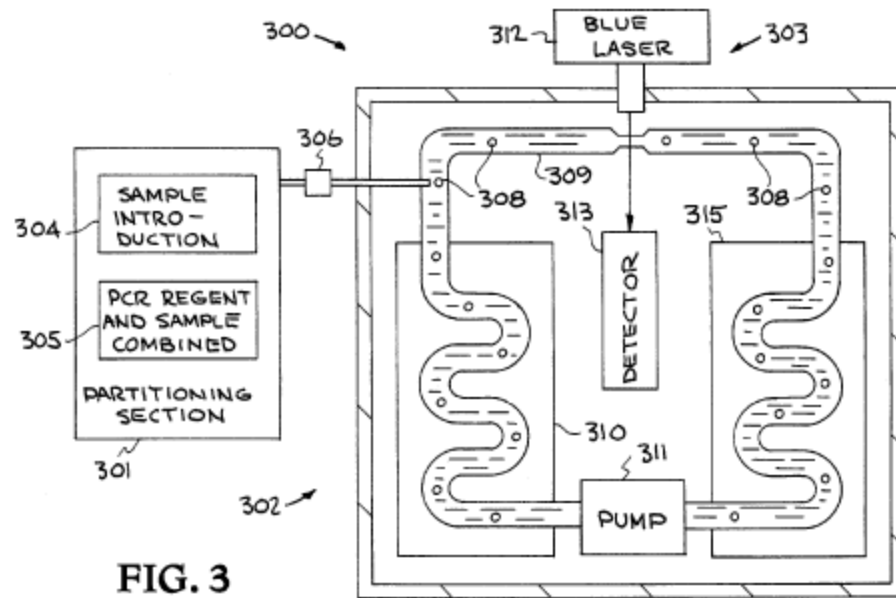


FIG. 3

2205. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” Anderson at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **308** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent)

through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Anderson at 6:41-7:8. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” Anderson at 7:47-50.

2206. It also would have been obvious to provide conditions suitable for PCR in the at least one plug such that the target DNA or RNA is amplified based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

2207. Claim 2 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2208. Claim 2 further recites: “**the step of providing conditions includes heating.**”

2209. Seki satisfies this limitation. For example, Seki discloses that “[a] temperature of the microchip is adapted to be controlled by a temperature controller.” Seki at [0126].

2210. While it is my opinion that Seki discloses providing heating to the microfluidic system, it also would have been obvious to combine the teachings of Seki with one or more prior

art references to satisfy this element. For example, Corbett discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett at 1:7-11. Corbett also describes that the invention involves “bringing the stream of carrier fluid containing the reaction mixture into contact with a plurality of different temperature zones, each temperature zone being held at a predetermined temperature and the stream of carrier fluid being in contact with each temperature zone for a predetermined period of time.” Corbett at 3:26-33.

2211. It also would have been obvious to provide heating to the microfluidic system in view of Lagally. Lagally describes small volume PCR and discloses, for example, that “[t]hermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.” Lagally at Fig. 1.

2212. It also would have been obvious to provide heating to the microfluidic system in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2213. It also would have been obvious to provide heating to the microfluidic system in view of Curcio. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid, Curcio at Paper V, 1. Curcio describes relatively small volume PCR that takes place in “plugs” in a continuous flow of immiscible organic liquid,

Curcio at Paper V, 1, including perfluorodecalin. Curcio at 9. The aqueous plugs comprise: genomic human placenta DNA, primers, ampliTaQ polymerase, and dNTPs in a “total reaction volume of approximately 300 nl” which “corresponded to a sample plug length of ca 1 cm.” Curcio at 5. The plugs were passed through three temperature zones: “denaturation at 94 °C, annealing at 54 °C and elongation at 72 °C.” Curcio at 5.

2214. It also would have been obvious to provide heating to the microfluidic system in view of Anderson. Anderson describes “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.” Anderson at Abstract. “In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. . . . These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**.” Anderson at 6:59-66.

2215. It also would have been obvious to provide heating to the microfluidic system based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

2216. Claim 3 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2217. Claim 3 further recites: “**providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.**”

2218. Seki satisfies this limitation. For example, Seki discloses that “when a microchip

involves a control mechanism for a trace quantity of liquid according to the second embodiment of the invention in a manner, for example, as described above, an analysis, a chemical reaction, or the like wherein a trace quantity of a sample is handled can be conducted. In this case, since the whole microchip is transparent, a variety of reactions of liquids introduced in the microchip can be easily observed.” Seki at [0143].

2219. While it is my opinion that Seki discloses providing a detector, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake also satisfies this limitation. I incorporate my analysis with respect to ¶¶ 1826-1830, demonstrating how Quake discloses a detector.

2220. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett discloses that “[i]n a preferred embodiment of the present invention there is provided an in-line analysis means downstream of the plurality of zones at differencing temperatures. The in-line analysis means determines the extent of amplification which has occurred in the reaction mixture and may additionally determine the specificity of amplification of defined target DNA sequence(s).

2221. It also would have been obvious to provide a detector based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 6*

2222. Claim 6 of the ’148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2223. Claim 6 further recites: “**the oil is fluorinated oil.**”

2224. Seki at least renders obvious this limitation, in light of the background knowledge

of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2225. It also would have been obvious to use a fluorinated oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2226. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2227. It also would have been obvious to use a fluorinated oil based on Seki in light of

the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 7*

2228. Claim 7 of the '148 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2229. Claim 7 further recites: “**the carrier fluid further comprises a surfactant.**”

2230. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2231. It also would have been obvious to use a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2232. It also would have been obvious to use a surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine

and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 8*

2233. Claim 8 of the '148 patent is dependent on claim 7. I incorporate by reference my analysis with respect to claims 1 and 7.

2234. Claim 8 further recites: “**the surfactant is fluorinated surfactant.**”

2235. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2236. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2237. It also would have been obvious to use a fluorinated surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XI.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2. *Motivation to Combine and Reasonable Expectation of Success*

2238. A POSA would have seen compelling reasons to modify the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR in small volumes as taught by Corbett, Lagally, Burns (1996), or Wang. This is because the prior art clearly taught that reactions could be conducted within microfluidic droplets, and there were numerous advantages associated with these microfluidic droplet reactors. In particular, a POSA would have considered it obvious to modify the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR based on numerous teachings in the art, including Corbett, Lagally, and Burns (1996), which discussed small-scale and even on-chip PCR (*see, e.g.,* Burns (1996)). A POSA would have had a reasonable expectation of success in so modifying, as evidenced by both the prior art and contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. Indeed, Quake itself describes both enzymatic reactions with biological molecules and PCR within microfluidic droplets. Quake at [0080] and [0170].

2239. A POSA would have been strongly motivated to perform PCR in the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki because doing so would have provided the substantial benefits known to be associated with microfluidic reactors. For example, Nisisako noted that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation, and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” *Treating liquid samples in droplet shape has the advantage that dead volume can be decreased.* Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is *likely to become increasingly important.*” Nisisako at 24 (emphasis added).

2240. A POSA would also prefer to carry out reactions in microfluidic droplets because its small dimensions allow for reduction of diffusion time for bimolecular reactions. Biomolecular reactions require two molecules to first encounter each other by diffusion or convection-enhanced diffusion. The reaction time and reaction yield for a given reactor are then determined by the diffusion time and then the kinetic time after the molecular encounter. By reducing the diffusion time, a micro-droplet reactor can significantly enhance the reaction yield. *See, e.g.,* Burns (2001) at 10. The reduction of the diffusion time also allows for careful analyses of different kinetic times or kinetic rates, thus allowing for the selection or screening of chemical or biological catalysts. If such reactions involve thermal programming, the low thermal capacitance of droplets also allows very rapid temperature change, thus preventing undesirable by-products.

2241. As another example, Lagally explained that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to *increase the speed of these assays* and to *reduce the amount of material and reagents needed*.” Lagally at 565 (emphasis added). Because PCR relies on reagents that are often in limited supply—for example, sample DNA—the ability to reduce both the amount of material needed for the reaction to occur and the dead volume of the reaction would have been highly motivating. Ferrance similarly explained that “[t]he same advantages of *reduced time, sample, and reagents* brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions.” Ferrance at 192

(emphasis added). The modification of Corbett, Lagally, Burns (1996), or Wang to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn would decrease operating costs. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

2242. Curcio likewise taught that “[m]inuturization of the fluidic system is beneficial in two ways: it *enhances the speed of thermal equilibration of the reaction mixture*, thus allowing increased flow velocities and faster PCR. Also *analyte volumes are reduced*, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level.” Curcio at 7 (emphasis added).

2243. Vogelstein additionally taught that microfluidic PCR enabled a sample to be diluted into thousands of discrete reaction volumes that each contained either one template PCR molecule or no DNA molecules. Vogelstein at 9236, 9239. A POSA would have found this advantageous because individual-template PCR reactions would have enabled the detection of relatively rare mutations, dislocations, and allelic imbalances. Vogelstein at 9236, 9239.

2244. Anderson further taught multiple advantages to a microdroplet PCR system. For example, Anderson described that:

Isolating the PCR reaction in such small (picoliter) volumes provides an order of magnitude reduction in overall detection time by:

(1) reducing the duration of each temperature cycle—the concentration of reactants increases by enclosing them in picoliter type volumes. Since reaction kinetics depend on the concentration of the reactant, the efficiency of a microdroplet should be higher than in an ordinary vessel (such a test tube) where the reactant quantity is infinitesimal

(2) reducing the total number of cycles—dilution of the fluorescently generated signal is largely eliminated in such a small volume, allowing much earlier detection. This effect is directly related to the number of microdroplets formed from the initial sample/reagent pool. Since PCR is an exponential process, for example, 1000 microdroplets would produce a signal 10 cycles faster than typical processing with bulk solutions.

(3) removing interference from competing DNA templates—given the extremely small volumes involved, it is possible to isolate a single template of the target DNA in a given microdroplet. A pL microdroplet filled with a 1 pM solution, for example, will be occupied by only one molecule on average. This makes it possible to amplify only one template in mixtures containing many kinds of templates without interference. This is extremely important in processing of real world aerosol samples containing complex mixtures of DNA from many sources, and has direct application in screening of precious cDNA libraries.

Anderson at 7:63-8:25.

2245. Reduction in size of the reaction vessel also allows for precise quantification of, for example, nucleic acids and pathogens. As a single template nucleic acid or pathogen can be placed in a droplet, detection of successful PCR amplification in a given number of droplets allows for digital quantification of, for example, the number of template nucleic acid or pathogens present. For the same reasons, other types of patterns, including *irregular* expression of nucleic acids, could also be quantified. A POSA would have expected that the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR that would enable these applications.

2246. Further, conducting PCR in microfluidic droplets would reduce potential contamination of the reaction, an issue that the prior art had recognized. *See, e.g.*, Corbett at 3:6-12 (“The most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by

small amounts of DNA which contain sequences capable of being amplified under the conditions of the assay.”).

2247. It was also well known that decreasing the scale of PCR to microfluidic levels provided the substantial advantage of making reactors portable. For example, Kopp explained that portable PCR microreactors could enable “[o]n-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Kopp at [1047]. Further, it was known that portable PCR reactors could aid physicians in the development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Kopp at [1047]. Thus, the prior art demonstrated that using the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to perform PCR would have advantageously allowed PCR to be performed in point of care diagnostic applications.

2248. Additionally, using the microfluidic reactors for PCR reactions would have substantially increased the tolerance of PCR reactions to primer non-specificity. As of the filing date, it was well known that PCR reactions suffered from the limitation that the primers were not always specific to the sequence of interest but rather could also bind to other sequences. Cha at 526. Because PCR amplification reactions are exponential in nature, PCR would often be ineffective where these other DNA fragments outnumbered the fragments of interest. *Id.* In such circumstances, the amplification products of the former would greatly exceed the amplification of the latter. *Id.* By using multiple droplets, a POSA could reduce the chances of having an uncontaminated DNA template in a single reaction. *Id.* Further, a POSA could conduct exponential amplification of the template without having the intended amplification product compete with unintended amplification products. *Id.*

2249. Moreover, a POSA would have expected the combination of microfluidic droplet reactors and PCR to be successful. For example, in 2001, Lagally et al. provided an overview of the evolution of continuous flow PCR microreactors:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample

volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Lagally at 565-566.

2250. In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct single-molecule DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

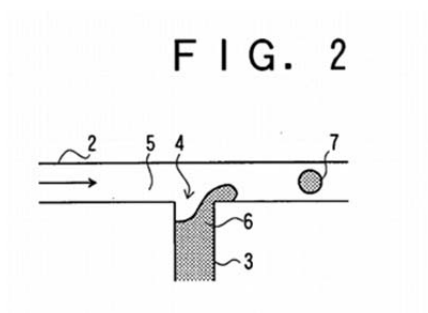
The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Lagally at 566-570. Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed

priority date. Consistent with the observations in the prior art, a POSA would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR reactions.

2251. The fact that several other groups simultaneously developed microfluidic systems that fall within the claims of the Ismagilov patents provides further evidence that a POSA would have both found the combinations described above obvious and would have had a reasonable expectation of success in so combining. For example, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001, more than a year prior to the '148 patent's earliest claimed priority date. *See* Higuchi I-III.

2252. Higuchi I discloses “a process and apparatus for rapidly producing an emulsion and microcapsules in a simple manner.” Higuchi I at Abstract. As an example, Higuchi describes that “[a] process for producing an emulsion includes a step of ejecting a dispersion phase from a dispersion phase-feeding port toward a continuous phase flowing in a microchannel in such a manner that flows of the dispersion phase and the continuous phase cross each other, whereby microdroplets are formed by the shear force of the continuous phase and the size of the microdroplets is controlled.” Higuchi I at [0006]. This is illustrated by Figure 2 in Higuchi I, reproduced below:



Higuchi I at Fig. 2. In the text accompanying the figure, and corresponding with the numbers, Higuchi I describes that “[a] dispersion phase (6) is ejected from a dispersed phase feeding port

(4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Abstract. The microfluidic droplet system Higuchi and his colleagues developed was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet systems to create droplets from continuously flowing streams of water and oil—and the use of those droplets to conduct reactions—was within the level of skill in the art as of the earliest effective priority date.

2253. As another example, Todd Thorsen (who co-authored the Thorsen reference discussed above) also developed a droplet reactor that falls within the claims of the Ismagilov patents. Thorsen Thesis at 94-108. The Thorsen Thesis describes the following microfluidic droplet reactor:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

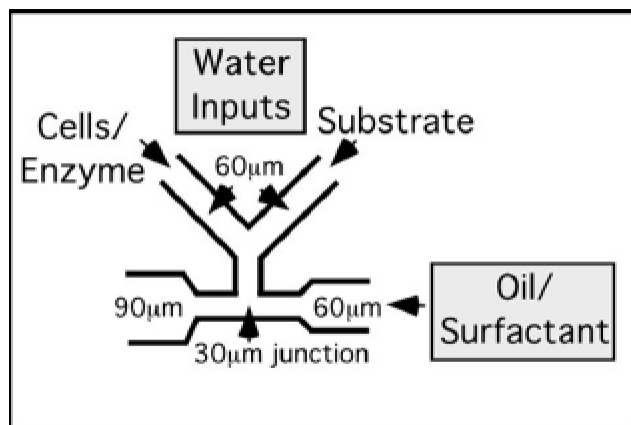


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

2254. The Thorsen thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002, suggesting that Thorsen’s work was performed before this date. The Thorsen Thesis was deposited with CalTech THESIS on December 2, 2002. Thorsen Thesis at 10X-000255686. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

2255. A POSA would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Schubert, or Krafft in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ramsey at 6:49-50 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). Krafft also noted that “the relatively low reactivity of fluorchemicals allows them to be combined with a wide variety of compounds without altering the properties of the incorporated agent.” Krafft at 2:27-30. For example, Curcio

described that perfluorodecalin was utilized as a carrier fluid with small-volume PCR because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity [and thus, high biocompatibility] towards biomolecules.” Curcio at 9. Therefore, a POSA conducting a PCR would have used fluorinated oils and fluorinated surfactants with these microfluidic droplet systems. For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

2256. Further, fluorinated oil offers high immiscibility with water and low solubility of biomolecules. *See, e.g.*, Schubert at 97 (“Fluorinated compounds also offer the potential for biomedical applications. For example, . . . fluorinated alkanes are . . . chemically and biologically stable.”); *id.* (“Because fluorocarbons are insoluble in water, however, they are currently administered in the form of emulsions, the formation of which requires the use of surfactants.”). Unlike most mineral oils, fluorinated oil has a density higher than water. Gelest at 19. This higher density allows easy separation of aqueous droplets from the oil when the emulsion is collected off the substrate.

2257. The art had also already noted that fluorination was preferable for silicon-based microfluidic devices, which have a tendency to swell when exposed to hydrocarbon oils. *See* Quake at [0118] (emphasis added) (“**TEFLON [which contains fluorination] is particularly suitable for silicon elastomer (RTV) channels**, which are hydrophobic and advantageously do not absorb water, but **they may tend to swell when exposed to an oil phase.**”). As Quake noted,

“[s]welling may alter channel dimensions and shape, and may even close off channels, or may affect the integrity of the chip, for example, by stressing the seal between the elastomer and a coverslip.” Quake at [0118]. This issue was also prevalent with PDMS, a silicon material that was commonly used to manufacture microfluidic substrates. *See* Quake at [0216] (emphasis added) (“In a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicon rubber or PolyDiMethylSiloxane (PDMS), ***preferably PDMS.***”); ’407 patent at 16:59-61 (“Channels may be molded onto optically transparent silicon rubber or polydimethylsiloxane (PDMS), ***preferably PDMS.***”).⁴⁵ Unlike other organic oils, fluorinated oil does not cause polymer like PDMS to swell. Holtze at 1632 (“In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.”) (citing Lee). Therefore, a POSA would have been motivated to use fluorinated oils and surfactants to prevent swelling of the polymer substrate.

2258. Importantly, fluorinated oil is far less viscous than other oils, including mineral oils. *See generally* Gelest. Instead, fluorinated oil has a viscosity similar to water. *Id.* Using a fluorinated oil with a microfluidic droplet device would thus allow high-frequency generation of droplets and parallel generation with multiple orifices. The prior art had already shown that high-throughput droplet generation was desirable. *See* Quake at [0079] (“This arrangement can be used to improve throughput or for successive sample enrichment, and can be adapted to provide a very high throughput to the microfluidic devices that exceeds the capacity permitted by conventional flow sorters.”); Quake at [0093] (“Monodisperse droplets may be particularly preferabl[e], e.g., in high throughput devices and other embodiments where it is desirable to

⁴⁵ I note that this language in the Ismagilov patents was copied almost directly from Quake.

generate droplets at high frequency.”). Further, the viscosity of fluorinated oil is insensitive to temperature, which is particular useful for DNA amplification reactions involving temperature changes. This of course includes PCR. Mullis at 9:55-60. For these reasons, a POSA would have been motivated to use fluorinated oil to achieve higher frequency droplet generation. Indeed, fluorinated oil has become the preferred carrier fluid for high-throughput aqueous droplet microfluidics. Autour at Section 4.1.

2259. As the prior art demonstrates, a POSA would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR as taught by Corbett, Lagally, Burns (1996), or Wang.

XII. INVALIDITY OF THE '091 PATENT

A. Summary of the '091 Patent

2260. The '091 patent is entitled “Device and Method for Pressure-Driven Plug Transport and Reaction.” The abstract explains that the invention “provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid.” '091 patent at Abstract.

2261. I understand that Bio-Rad is asserting claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent. Claims 1, 36, 37, and 57 are independent. Claims 2-3, 5-6, 11, 27, 29, 31, 33, and 35 depend on claim 1. Claims 38, 39, 43, 53, and 56 depend on claim 37. Claim 58 depends on claim 57.

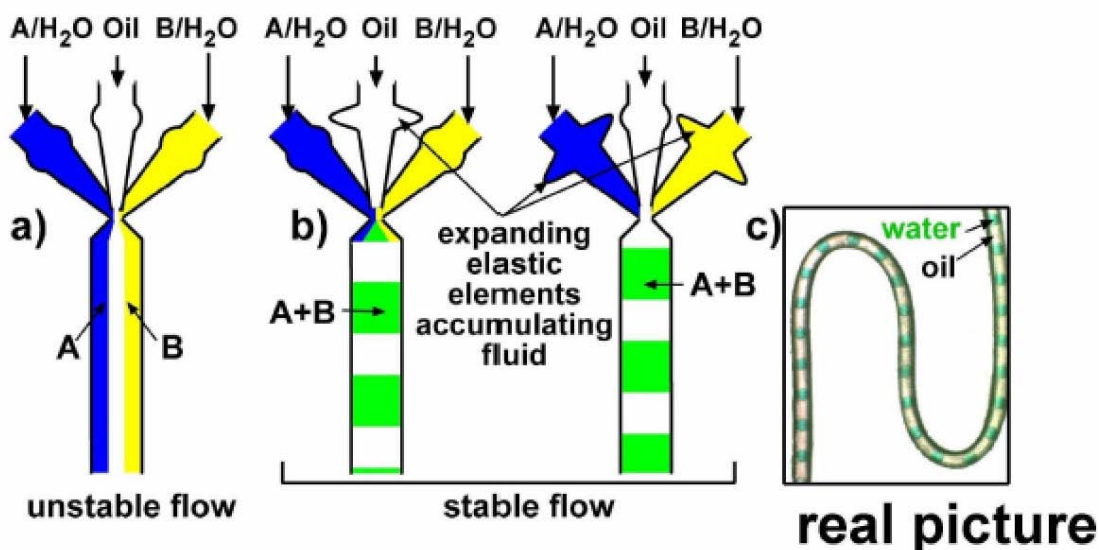
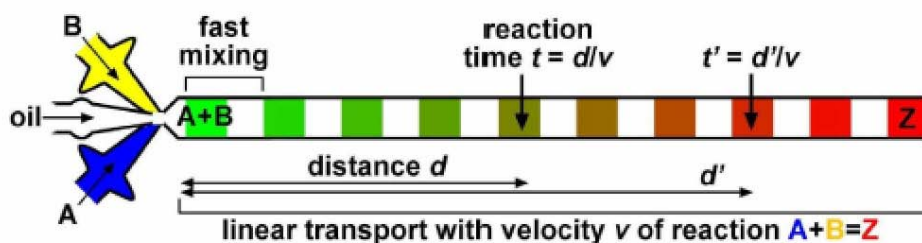
2262. The '091 patent issued from Application No. 10/434,970, filed May 9, 2003 (the “'970 application”). Provisional application No. 60/379,927 was filed May 9, 2002, and provisional application No. 60,394,544 was filed on July 8, 2002.

1. Priority

2263. I understand that Bio-Rad asserts that claims 1-2, 5-6, 11, 23, 29, 33, 35, 36, 37-39, 43, 53, 56-57, and 58 were conceived of “no later than October 16, 2001,” and relies on RI00111660–70 to support this assertion. Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 4. I further understand that Bio-Rad asserts that claim 3 was conceived of “no later than February 4, 2002,” and relies on RI00106817–18 to support this assertion, and that claims 27 and 31 were conceived of “no later than May 9, 2002,” and relies on U.S. Provisional Patent Application No. 60/379,927 (the “'927 provisional application”) to support this assertion. *Id.* I disagree with Bio-Rad’s assertions. The cited documents do not demonstrate that the inventors had formed in their minds a definite and permanent idea of complete and operative inventions as of the dates alleged.

2264. RI00111660–70, which Bio-Rad relies on to evidence conception of claims 1-2, 5-6, 11, 23, 29, 33, 35, 36, 37-39, 43, 53, 56-57, and 58, appears to be a portion of a PowerPoint presentation. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as October 16, 2001, but at best sets forth a research plan. While the first page of the presentation is dated October 16, 2001, the document’s metadata does not indicate when this PowerPoint was last modified or otherwise substantiate this date. Further, the document does not establish that the inventors had possession of every feature recited in claims 1-2, 5-6, 11, 23, 29, 33, 35, 36, 37-39, 43, 53, 56-57, and 58 or that every limitation of the claim was known to the inventors as of the date of this document. For example, all claims in the ’091 patent require “each plug” to be “substantially surrounded by carrier,” and this limitation is not suggested by any of the figures in RI00111660–70, which depict channels in with regions occupied by an “oil” and regions occupied by mixtures of aqueous fluids. The aqueous fluids do not appear to be substantially surrounded by oil. Instead, the aqueous fluid regions appear to be “slugs.”

Solution - true plug flow?



See, e.g., RI00111663. In fact, the slide suggests expanding elements are necessary to achieve stable flow. Unstable flow results when aqueous streams contact the main channel, which is indicative of a hydrophilic channel. This would suggest that the aqueous plug fluid can be in contact with the channel wall without significant encapsulation by the carrier fluid. Further, the channel layout depicted in these slides is not appropriate for plug formation. Instead of the T-junction approach later used in the Ismagilv patents, these slides depict a single oil channel between two aqueous channels. These channels do not intersect the main channel at an angle. This channel layout is also not appropriate for flow focusing as the two aqueous channels surround a single oil channel. The inverse would be used for flow focusing (two oil channels

surrounding a single aqueous channel). Based on my experience, this channel layout would not enable plug formation.

2265. As another example, each claim of the '091 patent requires "introducing" a "stream [or streams] plug fluid" and "introducing a carrier-fluid" to form "a plug." These limitations are not suggested by any of the slides in RI00111660–70, which do not depict or describe the formation of plugs from a "stream[s]" of aqueous fluid. Instead, as discussed above, the slides appear to depict "slugs" and the slides do not provide any indication that the aqueous fluids are "stream[s]."

2266. Similarly, for example, claims 5 and 39 recites "the method of claim [1 or 37], wherein the carrier-fluid comprises at least one surfactant," and this limitation is nowhere suggested in RI00111660-70, which generically describes the carrier fluid as "oil." Similarly, for example, claims 35 and 56 recite methods "wherein the volume of at least one plug is about 1 femtoliter to about 250nL," while RI00111660-70 does not suggest such limitations on the volumes of plugs.

2267. RI00106817-18, which Bio-Rad relies on to evidence the conception of claim 3, appears to be two lab notebook entries. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as February 4, 2002. As an initial matter, while the first entry bears the date February 4, 2002, the second entry does not, and Bio-Rad has identified no evidence corroborating its apparent assertion that either of these entries were created on February 4, 2002 (for example, these notes were not witnessed or countersigned by a third party). Further, the document does not establish that the inventors had possession of every feature recited in claim 3, or that every limitation of the claim was known to the inventor as of the date of the entry. For example, claim 3 depends

from claim 1, which requires a “reaction” of “reagents” to “substantially occur[]” in a plug, while the entry RI00106817 contains no references to any reactions. Instead, RI00106817 appears to refer to an experiment where streams of water with dye were introduced along with perfluorodecalin into a microchannel, with the observed result that “water and oil plugs were found to form consistently.” A reference in RI00106818 to “Fluorescence testing” is dated later than Bio-Rad’s claimed date of conception—February 5, 2002—and contains no references to reactions occurring in plugs.

2268. Bio-Rad relies upon the ’927 provisional application to evidence conception of claims 27 and 31. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as of May 9, 2002, and does not establish that the inventors had possession of every feature recited in claims 27 and 31 as of that date. This is discussed in more detail below, for example, because all of the deficiencies identified in the specification of the ’091 patent are also deficiencies of the ’927 provisional application.

2269. I understand that Bio-Rad asserts that claims 1-3, 5-6, 11, 23, 27, 29, 31, 33, 35, 36, 37-39, 43, 53, 56-57, and 58 were reduced to practice no later than May 9, 2002, based on the filing of the ’927 provisional application on that date. *See* Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 4. In my opinion, however, the ’927 provisional application fails to contain sufficient written description to establish that the inventor had possession of the alleged inventions claimed in the ’091 patent, and fails to enable the claims of ’091 patent. This is discussed in more detail below, for example, because all of the deficiencies identified in the specification of the ’091 patent are also deficiencies of the ’927 provisional application.

2270. I understand that Bio-Rad has provided no evidence of the inventors' diligence in reducing the alleged inventions of the '091 patent to practice after Bio-Rad's alleged dates of conception, and consequently that there is no evidence that any claim of the '091 patent would be entitled to priority as of Bio-Rad's alleged dates of conception, even if these dates were uncontested (which they are not). Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 4. Further, as set forth in **Exhibit 2**, I have reviewed various lab notebooks from Dr. Ismagilov's lab dated before May 9, 2003. None of these notebooks suggest that any work was done to reduce the inventions claimed in the '091 patent to practice in the three months between the alleged date of conception of claims 1-2, 5-6, 11, 23, 29, 33, 35, 36, 37-39, 43, 53, 56-57, and 58 (October 16, 2001) and January 16, 2002 (RI00106805). For example, none of these lab notebooks include experiments or work relating to conducting reactions in plugs before January 16, 2002.

2271. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception, diligence, or reduction to practice (and I understand that 10X's position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

B. Invalidity Overview

2272. As shown in further detail below, my opinions regarding the '091 patent include the following:

- All asserted claims are invalid under Section 112 for lack of proper written description and/or lack of enablement.
- All asserted claims are obvious in light of Quake under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Shaw Stewart under Section 103 (either alone or in combination with other references).

- All asserted claims are obvious in light of Burns (2001) under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Nisisako under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Thorsen under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Seki under Section 103 (either alone or in combination with other references).

C. Invalidity Under 35 U.S.C. § 112

2273. As described in further detail below, it is my opinion that the asserted claims of the '091 patent are invalid under 35 U.S.C. § 112.

1. *Written Description*

2274. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '091 patent are invalid for lack of written description.

2275. The claims of '091 patent, for example, require a "**reaction**." Bio-Rad appears to be taking the position that "**reaction**" is far broader than what was disclosed in the '091 patent. I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Based on Plaintiff's 4(c) disclosures, Bio-Rad contends that 10X performs a "DNA amplification reaction" in its 10X GemCode™ platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 7,129,091 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 10, 57, 64, 107, 136, 152, 161, 213, 220, 234, 244, 293, 300, 333, 345, 357, 403, 410; *see also, e.g.*, Appendix A to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 2 and 6. Further, based on Bio-Rad's Response to 10X's Interrogatory No.

4,

See, e.g., Appendix A to Plaintiffs’

Supplemental Response to Interrogatory No. 4 at 9-10. I have reviewed the ’091 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. The specification includes a single reference to a DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’091 patent at 46:2-5. There is no mention in the ’091 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. Indeed, there is no indication that the inventors of the ’091 patent contemplated any DNA amplification reaction beyond the basic (and well-known) PCR reaction. Nor has Bio-Rad identified any disclosure in the ’091 patent specification that discloses other DNA amplification reactions.

2276. There is also, for example, no adequate description of performing a “**reaction**” in plugs *outside of a substrate*, including, for example, a DNA amplification reaction in plugs outside of a substrate. I have reviewed the ’091 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. There is no indication that the inventors of the ’091 patent contemplated performing a DNA amplification reaction in plugs outside of the substrate. Nor has Bio-Rad identified any disclosure in the ’091 patent specification that discloses a DNA amplification reaction in plugs outside of the substrate.

2277. Bio-Rad has taken the following position:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can

be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, none of the identified reactions are DNA amplification reactions. Further, as discussed below, Bio-Rad has not identified any teaching in the ’091 patent that would convey to a POSA that the inventors had possession of a surfactant that would stabilize droplets and prevent droplet coalescence to allow for a “reaction” in plugs outside of the substrate, let alone a DNA amplification reaction in plugs outside of the substrate.

2278. None of the “embodiments in which droplets are captured in a capillary tube” (the “capillary tube embodiments”) identified by Bio-Rad, are included in the specification of the ’091 patent.

2279. In addition to the “capillary tube embodiments,” Bio-Rad has cites to a portion of the specification as “*contemplat[ing]* collection of droplets and removal from the chip,” First Supplemental Response to 10X’s Interrogatory No. 3 (emphasis added):

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

’091 patent at 17:23-30 (emphasis added). As an initial matter, this section “contemplates” collecting “*solution*” not *plugs* or *droplets*. Further, the specification provides no working

examples describing the collection of droplets in “a standard 1.5 ml centrifuge tube” or the “[c]ollection . . . using micropipettes”⁴⁶ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection, and subsequent DNA amplification outside of the substrate.

2280. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. However, as discussed below, the surfactant depicted in Figure 24 would not stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in plugs outside of the substrate.

2281. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927, these applications lack adequate description of performing a “**reaction**” including, for example, a DNA amplification reaction. . I have reviewed the ’544 and ’927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. The specifications of the ’544 and ’927 provisional applications do not include a single reference to a DNA amplification reaction. There is no mention in the ’544 or ’927 provisional applications of (for example) *any* DNA amplification reactions, let alone the details necessary to carry out said reactions. Nor has Bio-Rad identified any disclosure in the ’544 or ’927 specifications that discloses DNA amplification reactions.

⁴⁶ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

2282. The applications also lacks adequate description of a “**reaction**” outside of a substrate, including, for example, a DNA amplification outside of a substrate. I have reviewed the ’544 and ’927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the ’544 and ’927 proovisional applications that discloses a DNA amplification reaction in plugs outside of the substrate.

2283. Bio-Rad has taken the following position in its Response to 10X’s Interrogatory No. 3:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X’s Interrogatory No. 3.

2284. But the ’544 and ’927 provisional applications do not describe a single “embodiment in which droplets are captured in a capillary tube,” let alone a DNA amplification reaction in plugs outside of the substrate.

2285. The specifications of the ’544 and ’927 provisional applications state:

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted

for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'544 application at 28:22-26; '927 provisional application at 27:14-23 (emphasis added). Again, this section speaks to collecting “*solution*” not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in “a standard 1.5 ml centrifuge tube” or “[c]ollection . . . using micropipettes,”⁴⁷ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection.

2286. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The '544 and '927 provisional applications do not include this figure, or any related discussion. The '544 and '927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” '927 provisional application at 12:16-17; '544 application at 12:19-13:3,⁴⁸ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80).

⁴⁷ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

⁴⁸ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application at 10:31-11:15.⁴⁹ However, as discussed below, none of the surfactants described would stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets off the substrate.

⁴⁹ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 ("Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the "Span" surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug."); *see also* Quake at [0095]

2. *Enablement*

2287. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that the claims of the '091 patent are invalid for lack of enablement.

2288. The claims of the '091 patent, for example, require a "**reaction**." I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Based on Plaintiffs' 4(c) disclosures, Bio-Rad contends that 10X performs a "DNA amplification reaction" within plugs. Infringement of U.S. Patent No. 7,129,091 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 152, 234, 345 ; *see also, e.g.*, Appendix A to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 2 and 6. Further, based on Bio-Rad's Response to 10X's Interrogatory No. 4,

See, e.g., Appendix A to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 9-10. But the specification of the '091 patent does not enable the full scope of the limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. The claims purport to cover **all** DNA amplification reactions in plugs (whether known or unknown at the time of Ismagilov's alleged invention), but the specification includes a single reference to a DNA amplification reaction: "Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences." '091 patent at 46:2-5. The specification does not include a working example of a PCR reaction in plugs. And there is no mention in the '091 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. The '091 patent fails to disclose, teach, or suggest how to conduct every

“DNA amplification reaction,” and particularly, the “DNA amplification reactions” allegedly performed by 10X,⁵⁰ within plugs.

I understand that these techniques were developed by 10X years after the priority date of the Ismagilov patents.

2289. As another example, claims of the '091 patent, for example, require a “**reaction**.” I understand the Court has construed “reaction” as: “Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate.” Claim Construction Order at 1. Based on Plaintiffs’ 4(c) disclosures, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 7,129,091 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1, 10, 57, 64, 107, 136, 152, 161, 213, 220, 234, 244, 293, 300, 333, 345, 357, 403, 410. ; *see also, e.g.*, Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 2 and 6. Further, based on Bio-Rad’s Response to 10X’s Interrogatory No. 4,

See, e.g.,

Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 9-10. The claims purport to cover *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov’s alleged inventions), including DNA amplification reactions in plugs *outside of the substrate*. But the specification of the '091 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. The specification does not enable

⁵⁰ I have not been asked to provide, and have not formed an opinion on whether or not the reactions performed in 10X’s products are “DNA amplification reactions.”

DNA amplification reactions in plugs *outside of the substrate*. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. Surfactants that would enable a POSA to conduct biological reactions within plugs outside of the substrate, let alone DNA amplification reactions in plugs outside of the substrate, are not described in specification of the '091 patent and were not even available as of the alleged priority date of the '091 patent. In fact, surfactants appropriate for this use were not developed or described until 2008—seven years after Ismagilov's alleged invention.

2290. As discussed above, Bio-Rad contends that 10X performs a “DNA amplification reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.”

2291. The surfactants disclosed in the Ismagilov patents would not stabilize droplets under these conditions.

2292. In order to conduct biological assays within microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, preventing coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Holtze at 1632.⁵¹ “These requirements [were] particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluortelomer-tails (typically perfluorinated C₆ to C₁₀) . . . do not provide sufficient long-term emulsion stability.” *Id.*⁵² Even as of 2008, years after the priority date of the ’091 patent, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.*

2293. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. But none of the surfactants disclosed in the specification of the ’091 patent, including the surfactants disclosed in Figure 24, meet the requirements set forth above.

2294. As set forth in the specification, “FIG.24 shows a reaction scheme that depicts

⁵¹ Holtze was authored by individuals from Harvard University, Universit`a del Salento, Lecce, Italy, and Raindance Technologies, Inc. Holtze at 1632.

⁵² When conducting biological assays in droplets, “it is attractive to use a fluorocarbon oil as the continuous phase” and accordingly, a fluorosurfactant to “ensur[e] that drops are stable.” Holtze at 1632.

examples of fluorinated surfactants that form monolayers that are: (a) resistant to protein adsorption; (b) positively charged; and (c) negatively charged. Fig. 24b shows a chemical structure of neutral surfactants charged by interactions with water by protonation of an amine or guanidinium group. FIG 24c shows a chemical structure of neutral surfactants charged by interactions with water deprotonation of a carboxylic acid group.” ’091 patent at 5:32-40.

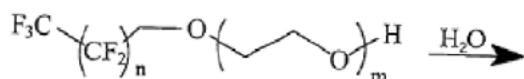


FIG. 24A

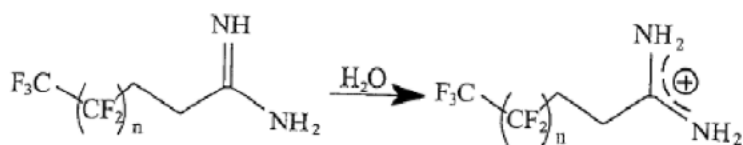


FIG. 24B

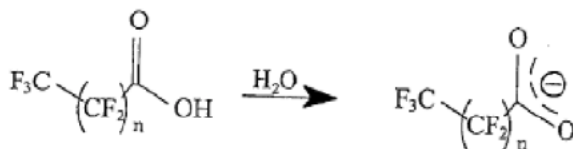


FIG. 24C

Fig. 24

2295. Specifically, Figure 24a “depicts a “fluorinated surfactants containing perfluoroalkyl chains [(red)] and an oligoethylene glycol head group [(blue)].” ’091 patent at 74:18-20.

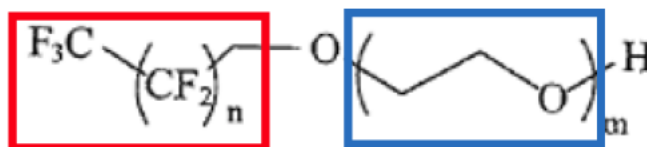


FIG. 24A

2296. The surfactant depicted in Figure 24a is commercially available under the trade

name Zonyl.” See ’091 patent at 20:62-64 (“Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)”); ’407 patent at 76:64-66 (“A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283.”).

2297. Figure 18, depicts the same fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group. ’091 patent at 58:40-42 (“In FIG. 18, plugs are formed in the presence of several solutions of surfactants that possess different functional groups (left side of the diagram)”) (annotation added).

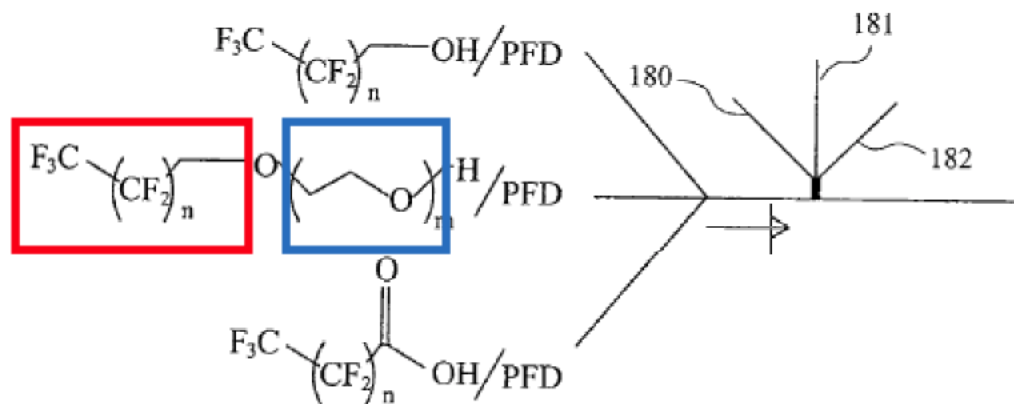


Fig. 18

2298. Unlike the ionic surfactants depicted in Figure 24b and 24c, the surfactant depicted in Figure 24a meets the second requirement set forth above. It will “produce a biologically inert interior surface for the water drops.” As described in the specification of the ’091 patent: “[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.” ’091 patent at 36:48-51. Further, this OEG head group is non-ionic as required for biological assays. Holtze at 1632. But this surfactant does not meet the first requirement set forth above for performing biological assays in

droplets. Specifically, it would not “provide stability to the drops, preventing coalescence.”

2299. The surfactant depicted in Figure 24a contains “a “perfluoroalkyl chain[] and an oligoethylene glycol head group.” ’091 patent at 74:19-20. A perfluoroalkyl chain (also referred to as a “perfluoroalkyl tail” of “fluorotelomer-tail”) is not sufficient to stabilize droplets outside of the substrate. As described by Holtze *et al.* “[s]urfactants with short fluorotelomer-tails” like the perfluoroalkyl chain depicted in Figure 24a, “do not provide sufficient long-term emulsion stability.” Holtze at 1632.

2300. I understand that Dr. Jeremy Agresti, Bio-Rad’s R&D Director and a co-author on Holtze *et al.*, confirmed this point. Dr. Agresti was questioned regarding the text copied below from Holtze *et al.*:

However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C₆ to C₁₀) have been used, but do not provide sufficient long-term emulsion stability. Fluorosurfactants with longer fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity.

Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632 (internal citations omitted).

2301. Dr. Agresti confirmed that these statements were accurate at the time they were written in 2008. Agresti Tr. 199:9-13 (“Q. Do you believe that the statements that are made in the article that you just read, those portions that the article that you just read, do you believe those are accurate? A. Yeah, at the time for sure.”).

2302. Further in reference to the following statement in Holtze et al.: “Biological assays thus demand fluorosurfactants with non-ionic head groups; however, there are currently no such surfactant available,” Dr. Agresti confirmed that as of 2008 there were no “fluorosurfactants with nonionic head groups that would stabilize and emulsion long term.” Agresti Tr. 202:2-13 (“Q. And it was true that as of – as of the date of this article, which was 2008, that at least to your knowledge that there were no nonionic fluorosurfactants with nonionic head groups? A. That could stabilize an emulsion long term. We knew that there were fluoro surfactants with nonionic head groups. Q. [W]hat was not known was that there were fluoro surfactants with nonionic head groups that would stabilize an emulsion long term. A. Yes, that’s right.”). Dr. Agresti further confirmed that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, specifically Zonyl, “doesn’t stabilize droplets for PCR.” Agresti Tr. 203:10-19 (Q. Are you familiar with a surfactant known as . . . ZONYL? A. Yes. Q. Has Bio-Rad used that surfactant? A. I can’t say. It’s not in any product. As far as I know it’s never been in any product. Q. Why not? A. As far as I know it doesn’t stabilize droplets for PCR.”).

2303. I understand that named inventor of the ’083 patent Mr. Lewis Spencer Roach, who testified that his “primary contribution” was to developing “fluorinated surfactant[s] with hydrophilic head group” Roach Tr. 26:21-23, also confirmed the point that a surfactant with a

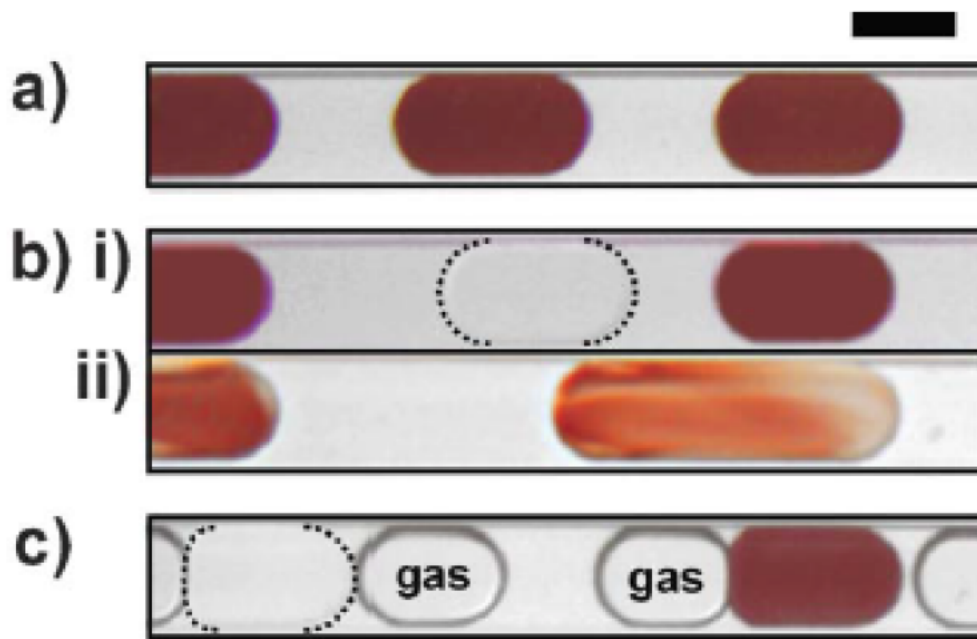
perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl would, not stabilize droplets long term. Mr. Roach testified that “other groups have done a lot of work on preventing coalescence using surfactants” but “I did not personally perform that research.” Roach Tr. 78:15-20. When asked whether “other groups” mean “other people in Dr. Ismagilov’s lab,” Mr. Roach answered that he “believe[d] it was outside of Ismagilov’s group.” Roach Tr. 78:21-79:2. Mr. Roach further testified that the “Rf-OEG surfactant is not optimized for preventing coalescence . . . [t]here are other hydrophilic head groups that are better at controlling adsorption than a simple oligo (ethylene glycol) head group. I think other people have made these.” Roach Tr. at 79:3-12.⁵³ Mr. Roach later confirmed that the “other people” he was referring to were Holtze et al. in 2008. Roach Tr. 80:4-11 (“A. I believe [Exhibit 129 (Holtze et al.)] is what I was just referring to, that other groups had optimized surfactants to – give me just a second. I want to read the conclusions in this paper here. Q. Certainly. A. Yes. This is where I was discussing other groups that have optimized surfactants to prevent coalescence or merging of plugs.”).

2304.

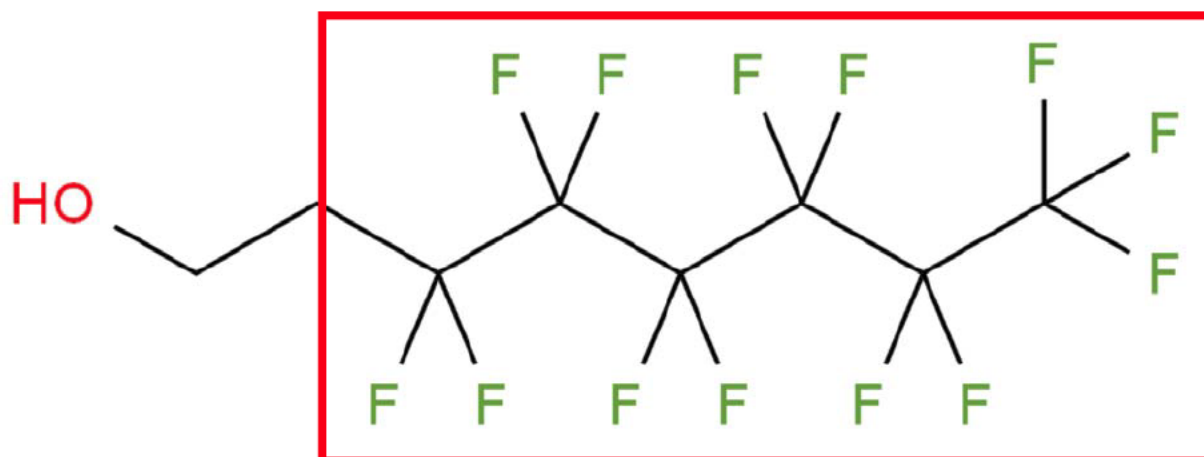
⁵³ The “Rf-OEG” surfactant is a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group. *See* Roach Depo. Ex. 127 (“Perfluorinated-tail, oligoethylene glycol derivatized molecules (Rf-OEG) were selected as a neutral and hypothetically biocompatible surfactants.”); Roach Tr. 51:22-23 (“A. I synthesized the surfactant described in [Exhibit 127], particularly the Rf-OEG surfactant.”).

2305. I agree with Agresti's, Roach's, and Hindson's statements above regarding fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl. Such surfactants would not provide stability to drops and prevent coalescence to allow for DNA amplification reactions in microfluidic droplet outside of the substrate .

2306. Dr. Ismagilov himself recognized the potential for coalescence, even between plugs within the substrate. As explained by Dr. Ismagilov, "[d]uring flow, plugs with different chemical composition may move relative to the carrier fluid at different rates and thus move relative to one another allowing adjacent plugs to coalesce (Fig. 2(b))." Adamson at 1181.



2307. Figure 2b above depicts plug coalescence between “[p]lugs of distinct chemical composition.” Adamson at 1181. The carrier fluid is “FC-3283 10:1 PFO (v/v) throughout.” Adamson at 1181. FC-3283 is a fluorinated oil. PFO or 1H,1H,2H,2H-perfluorooctanol is a fluorinated surfactant. The chemical formulation of PFO is depicted below:

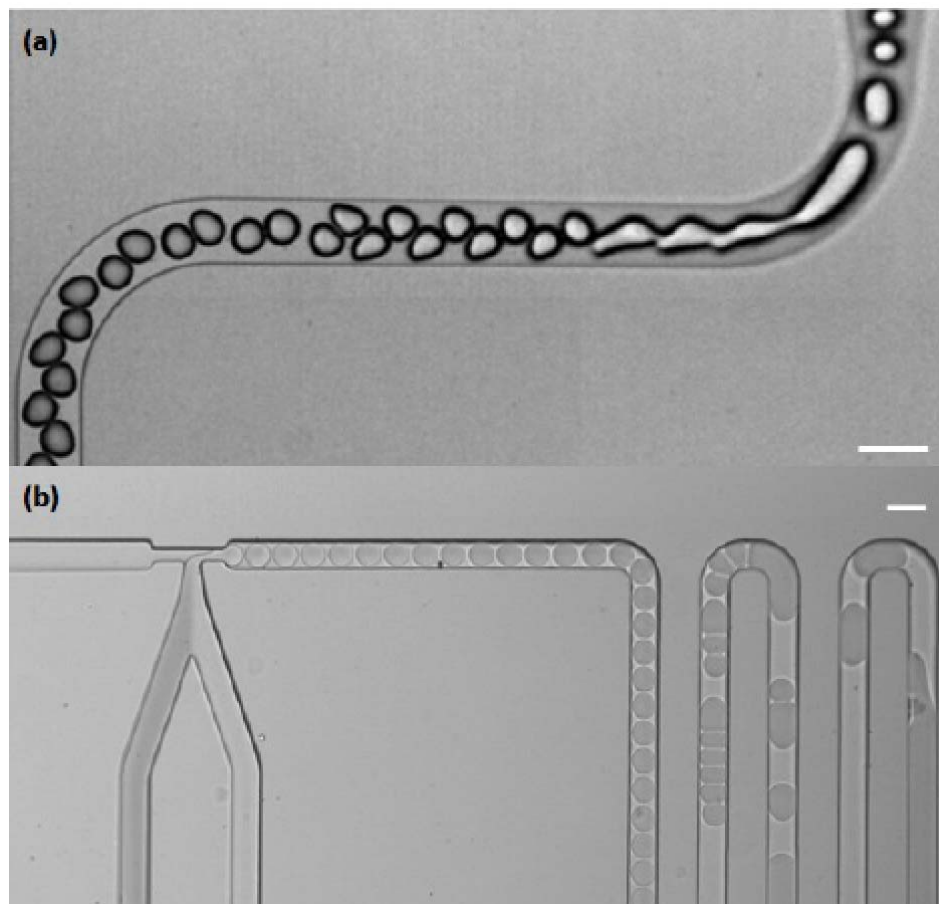


2308. Like Zonyl, PFO contains a short perfluoroalkyl tail (red).

2309. As explained by Dr. Ismagilov, this surfactant was insufficient to prevent

coalescence even within the substrate. *See* Adamson at 1181. Instead “[t]o prevent coalescence, gas bubbles [were] introduced as spacers between plugs to (1) minimize the relative motion of plugs and (2) to act as a physical barrier to prevent coalescence of adjacent plugs during flow and splitting.” Adamson at 1181.

2310. This potential for droplet coalescence was later described as “[u]ncontrollable.” Cho Thesis at Fig. 3.1. This thesis explained: “Perfluorodecalin and 1H,1H,2H,2H-perfluoro-1-octanol combination was used for studying protein crystallization by Ismagilov and his coworkers. The perfluorinated oil and surfactants are advantageous for microdroplet based biochemical applications as they are lipophobic, inert, insoluble in water and compatible with many biochemical molecules. Unfortunately, droplets in perfluorodecalin oil with 1H,1H,2H,2H-perfluoro-1-octanol were not stable and merged with each other under pressure (Figure 3.1(b)).” Cho Thesis at 50. The “[d]roplet generation and uncontrolled coalescence of droplets in perfluorodecalin with 5% v/v 1H, 1H, 2H, 2H-perfluoro-1-octanol” observed is depicted in (b) below:



2311. “Fluorosurfactants with longer fluorocarbon tails” are required for “long-term stabilization” to perform biological assays. Holtze at 1632. As further described by Holtze et al., as of 2008, no such surfactant existed in 2008:

However, the only available PFPE-based surfactants have ionic headgroups, e.g. poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity.¹⁵ Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632. Holtze et al. disclose examples of fluorinated surfactants meeting the two requirements set forth above. These surfactants comprise non-ionic polyethylene glycol head

groups and perfluorinated polyether tails. Holtze at 1; Figure 2.

2312.

2313. I understand that RainDance's droplet products utilize a "biocompatible surfactant, PEG-PFPE block copolymer." Plaintiffs' First and Second Supplemental Response to Interrogatory No. 5. Bio-Rad's droplet products utilize "Krytox K225 (0.58mM) + perfluorodecanol (0.625 mM) or the BRDG3 triblock fluorosurfactant." Plaintiffs' Third and Fourth Supplemental Response to Interrogatory No. 5.

2314. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927, the specifications of the '544 and '927 provisional applications do not enable a person of skill in the art to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate without undue experimentation. Surfactants necessary to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate are not described in specification of the '544 or '927 provisional applications and were not even available as of the filing date of the '544 or '927

provisional applications.

2315. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The ’544 and ’927 provisional applications do not include this figure, or any related discussion. The ’544 and ’927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” ’927 provisional application at 12:16-17; ’544 application at 12:19-13:5,⁵⁴ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or

⁵⁴ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application at 10:31-11:15.⁵⁵ In the context of conducting biological assays in microfluidic droplets outside of the substrate, each of the surfactants listed—excluding “fluorinated oil” discussed separately below—would be considered an aqueous soluble surfactant by a POSA, meaning they are introduced in the aqueous phase instead of the oil phase. To conduct biological assays in microfluidic droplets outside of a substrate, a POSA would understand that a continuous phase comprised of a fluorinated oil is preferred, if not necessary. Holtze at 1632. The listed surfactants are non-fluorinated and as such are not soluble in fluorinated oil. Therefore, to use one of the listed surfactants in a system comprising a fluorinated oil, the surfactant needs to be introduced into the aqueous phase. However, when present in the aqueous phase these surfactants would be disruptive to emulsion stability. The hydrophobic portions of these surfactant molecules cause them to populate the aqueous-fluorinated oil droplet boundary, displacing any stabilizing fluorinated surfactant molecules present. This process leads to droplet coalescence rather than stabilization.

⁵⁵ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [*sic*] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

2316. “Fluorinated oil” while soluble in fluorinated oil, also would not stabilize droplets to conduct biological assays in microfluidic droplets off of the substrate. Holtze at 1632 (“[I]t is attractive to use fluorocarbon oil as the continuous phase However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable.”).

D. Invalidity Based on Prior Art

1. Obviousness

(a) Invalidity Based on Quake

2317. It is my opinion that Quake discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) Claim 1

2318. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2319. I understand that the Court has not considered whether the preamble of this claim is limiting.

2320. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

2321. Quake describes that, in some embodiments, the droplets created in the

microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

2322. Quake even describes a specific type of chemical reaction involving enzymes produced by cells:

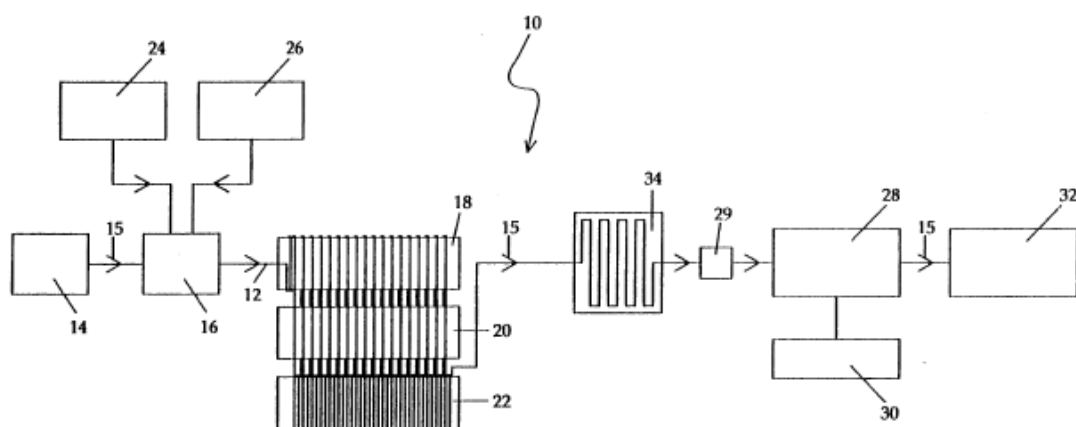
In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

2323. While it is my opinion that Quake discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2324. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material

and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2325. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2326. It also would have been obvious to conduct a reaction within at least one plug in

view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2327. It also would have been obvious to conduct a reaction within at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2328. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2329. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or*

flow of oil in a main channel of the device.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2330. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2331. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2332. Quake at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example,

Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a *pressurized stream of oil* is passed, and at least one sample inlet channel, through which a *pressurized stream of aqueous solution* is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a pressurized stream or flow of oil in a main channel of the device.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2333. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as *an ‘extrusion’ or ‘barrier’ fluid*, passes (i.e., flows) through the main channel of the device and a *second fluid, referred to as a ‘sample’ or ‘droplet’ fluid*, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2334. Quake also describes different droplet-formation configurations. For example,

Quake discloses that “[i]n another preferred embodiment, the device of the invention comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a first droplet extrusion region, and a second inlet region in communication with the main channel at a second droplet extrusion region.” Quake at [0018]. Quake also describes that “[i]n the preferred embodiment illustrated in Fig. 22, the first inlet channel 2202 may introduce an aqueous solution containing an enzyme so that aqueous droplets containing molecules of the enzyme are introduced into the stream of oil in the main channel 2201. The second inlet channel 2203 may introduce an aqueous solution containing a substrate for the enzyme so that aqueous droplets containing molecules of the substrate are also introduced into the main channel 2201. In more detail, droplets containing the enzyme are first sheared off into the main channel 2201 at the first droplet extrusion region 2204. These droplets then move downstream, with the oil stream in the main channel, and pass through the second droplet extrusion region 2205.” Quake at [0316]. Figure 22 from Quake is reproduced below:

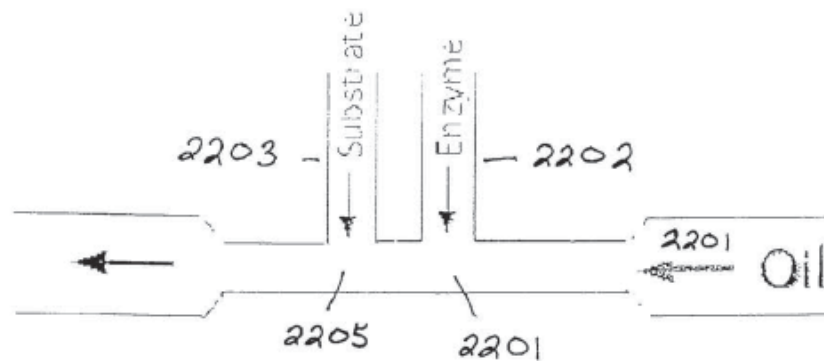


FIG. 22

Quake at Fig. 22. Quake states that “[a]lthough the exemplary embodiment described here, and illustrated in FIG. 22, releases droplets of enzyme upstream from the droplets of substrate,

droplets of the different fluid or solutions may be released in any order. Thus, for example, an aqueous solution containing a substrate may be released through the first inlet channel 2202 at the first droplet extrusion region 2204, and droplets of an aqueous solution containing an enzyme may be released through the second inlet channel 2203 at the second droplet extrusion region 2205.” Quake at [0318].

2335. Quake also describes that “in preferred embodiments, the sample inlet and main channel intercept at a T-shaped junction; i.e., such that the sample inlet is perpendicular (90 degrees) to the main channel. However, the sample inlet may intercept the main channel at any angle . . . Other shapes and channel geometries may be used as desired.” Quake at [0084]-[0085]. Indeed, this language was copied almost exactly in Ismagilov’s 60/379,927 provisional application, to which the ’091 patent claims priority. *See* Ismagilov ’927 provisional application at 9:31-39.

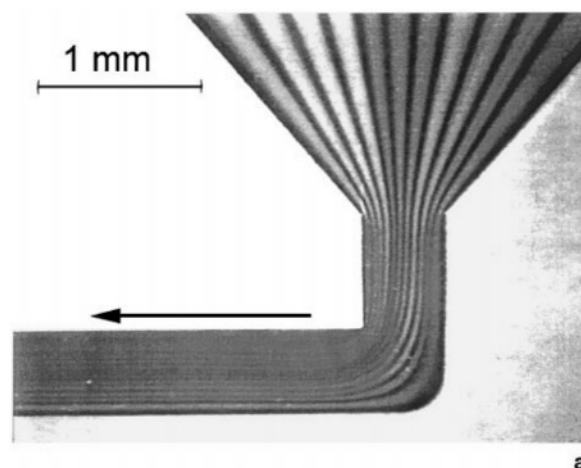
2336. It also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2337. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during

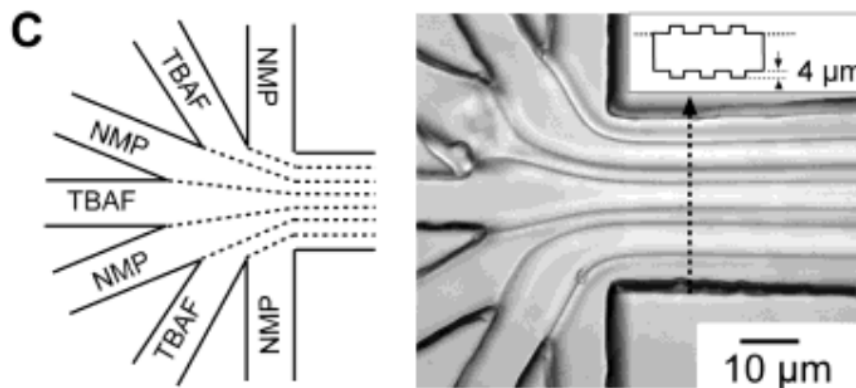
parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2338. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2339. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2340. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



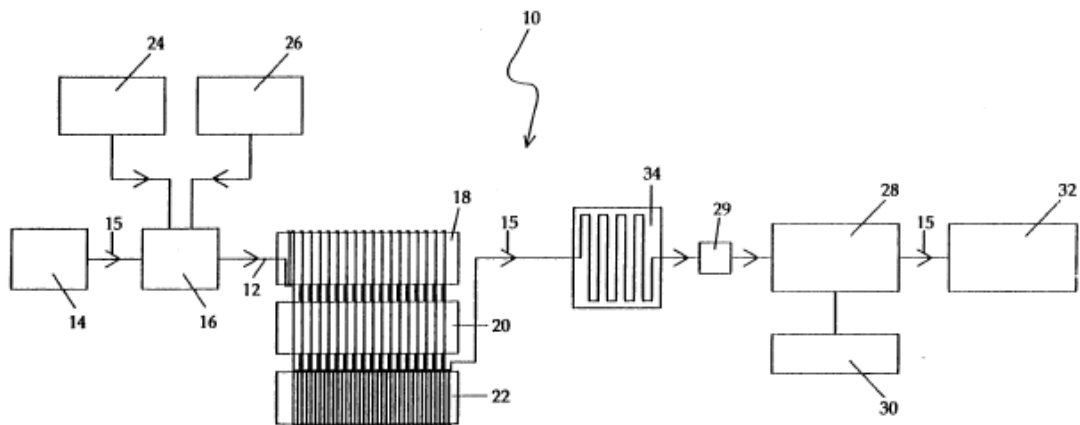
Whitesides at 845-846.

2341. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2342. While it is my opinion that Quake discloses that each plug comprises reagents so

that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

2343. It also would have been obvious that each plug comprises reagents so that a

reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2344. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into

the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2345. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

2346. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2347. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

2348. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

(ii) *Claim 2*

2349. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2350. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

2351. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within *microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0014] (“In a preferred embodiment, water droplets are extruded into a flow of oil”); Quake at [0015] (“For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).”); Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane or hexadecane)”; Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane)”))

2352. Quake also explains that the carrier fluid, or “extrusion fluid,” is “incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel.” Quake at [0022].

2353. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

(iii) *Claim 3*

2354. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my

analysis with respect to claim 1.

2355. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

2356. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*”).

2357. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

2358. While it is my opinion that Quake discloses a carrier-fluid comprising a fluorinated compound, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2359. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2360. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2361. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

2362. Claim 5 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2363. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2364. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

2365. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel . . . The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

2366. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating

which minimizes adhesion . . . Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

2367. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(v) *Claim 6*

2368. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2369. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

2370. Quake satisfies this limitation. For example, Quake describes that the sample fluid used with his devices and methods can be an “aqueous fluid”: “The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, *through which a pressurized stream of aqueous solution is passed.*” Quake at [0003] (emphasis added).

(vi) *Claim 11*

2371. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2372. Claim 11 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

2373. Quake satisfies this limitation, describing a specific kind of reaction with a

soluble reaction product:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

(vii) *Claim 27*

2374. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2375. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

2376. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); Quake at [0015] (“For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (*e.g.*,

tetradecane or hexadecane) or another oil (for example, mineral oil).”); Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane or hexadecane)”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane)”). Quake therefore disclosed that different oils could be used to form the carrier fluid.

2377. A POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* ’091 Patent at Table 1 (refractive index of water is 1.3330) to Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

(viii) *Claim 29*

2378. Claim 29 of the ’091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2379. Claim 29 further recites: “**employing a number of devices in parallel.**”

2380. I understand that Bio-Rad is contending that “microfluidic chips with eight channels in parallel” fall within the scope of this claim. *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Quake satisfies this limitation. For example, Quake states that “[a]n ‘analysis unit’ is a microfabricated substrate, e.g., a micro fabricated chip, having at least one inlet region, at least one main channel, at least one detection region and at least one outlet region . . . ***A device according to the invention may comprise a plurality of analysis units.***” Quake at [0067].

(ix) *Claim 31*

2381. Claim 31 of the ’091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2382. Claim 31 further recites: “**the reaction is a polymerization reaction.**”

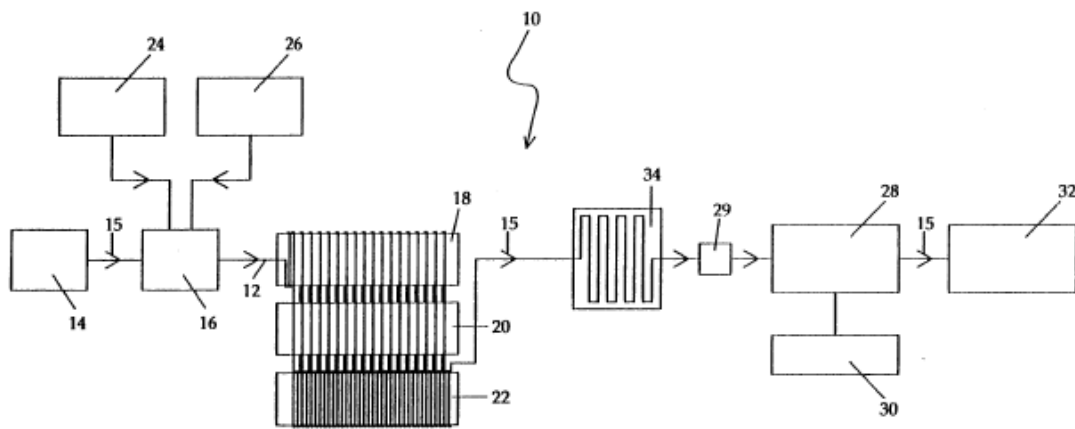
2383. Quake satisfies this element. For example, Quake discloses that

“[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

2384. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Quake discloses this limitation.

2385. While it is my opinion that Quake discloses a polymerization reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2386. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2387. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2388. It also would have been to conduct a polymerization reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

2389. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2390. Claim 33 further recites: “**each plug initially has a cross section that is**

substantially the same size as the cross section of the channel at the inlet.”

2391. Quake satisfies this limitation. For example, Quake discloses that “[i]n one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes.” Quake at [0092].

2392. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

2393. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2394. Claim 35 further recites: **“the volume of at least one plug is about 1 femtoliter to about 250 nL.”**

2395. Quake satisfies this limitation. For example, Quake discloses that “[i]n preferred embodiments, the droplets of aqueous solution have a volume of approximately 0.1 to 100 picoliters (pL).” Quake at [0003].

2396. Quake also explains that “[f]or particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are preferably rounded, with a diameter between 2 and 100 microns, preferably about 60 microns, and more preferably about 30 microns at the crossflow area or droplet extrusion region . . . Similarly, the volume of the detection region in an analysis device is typically in the range of between about 10 femtoliters (fL) and 5000 fL, preferably about 40 or 50 fL to about 1000 or 2000 fL, most preferably on the order of about 200

fl. In preferred embodiments, the channels of the device, and particularly the channels of the inlet connecting to a droplet extrusion region, are between about 2 and 50 microns, most preferably about 30 microns.” Quake at [0091].

2397. Quake also provides a formula that can be used to calculated droplet size:

The size of a droplet in a micro fluidic device of this invention may be provided by the equation:

$$r = \frac{\sigma}{\eta \varepsilon}$$

where r is the final droplet radius in a main channel. η , the viscosity of the continuous phase (e.g., the oil-surfactant phase in the above exemplary devices) and σ , the interfacial tension, may be obtained from values available in the art for the particular fluids used (see, for example, CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Fla., 2000). ε , which denotes the shear rate, may be provided by the formula

$$\varepsilon = \frac{2}{y_0} v,$$

where v is the velocity of the dispersed phase fluid (i.e., the droplets) and may be readily calibrated to the input pressures for a particular microfluidic device. Y_0 denotes the radius of the inlet channel at the droplet extrusion region (i.e., the radius of the tapered channel 1606 in FIG. 16B).

Quake at [0308]-[0310].

(xii) *Claim 36*

2398. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2399. I understand that the Court has not considered whether the preamble of this claim is limiting.

2400. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

2401. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

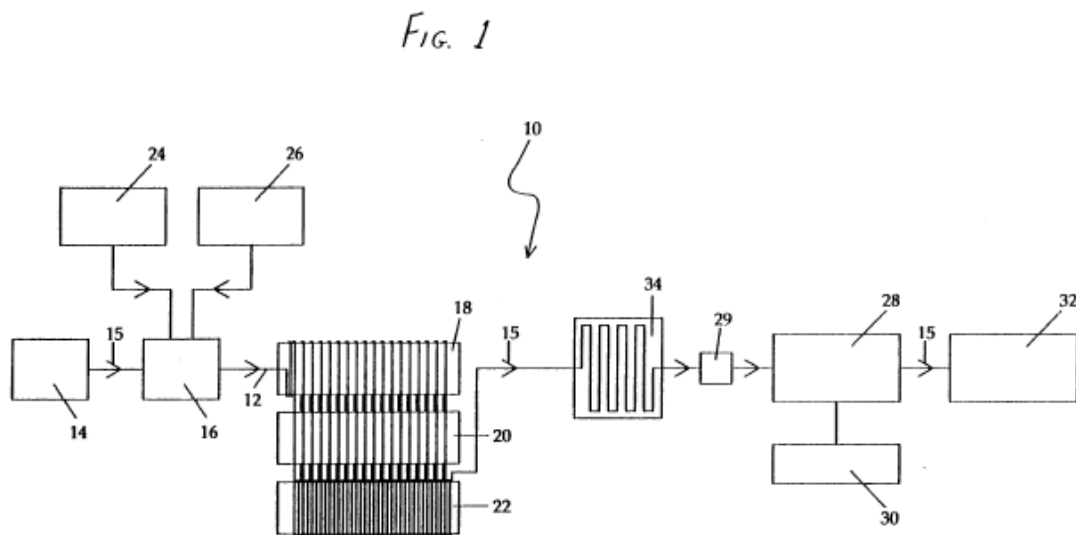
2402. Quake even describes a specific type of chemical reaction involving enzymes produced by cells:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

2403. While it is my opinion that Quake discloses a method for conducting a reaction

within at least one plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2404. It also would have been obvious to conduct a reaction within at least one plug in

view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2405. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.”

Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2406. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2407. It also would have been obvious to conduct a reaction within at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2408. Claim 36 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2409. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of

aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device*.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2410. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2411. Claim 36 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first**

microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

2412. Quake at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a *pressurized stream of oil is passed*, and at least one sample inlet channel, through which a *pressurized stream of aqueous solution* is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a pressurized stream or flow of oil in a main channel of the device.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2413. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as *an ‘extrusion’ or ‘barrier’ fluid*, passes (i.e., flows) through the main channel of the device and a *second fluid, referred to as a ‘sample’ or ‘droplet’ fluid*,

passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2414. Quake also describes different droplet-formation configurations. For example, Quake discloses that “[i]n another preferred embodiment, the device of the invention comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a first droplet extrusion region, and a second inlet region in communication with the main channel at a second droplet extrusion region.” Quake at [0018]. Quake also describes that “[i]n the preferred embodiment illustrated in Fig. 22, the first inlet channel 2202 may introduce an aqueous solution containing an enzyme so that aqueous droplets containing molecules of the enzyme are introduced into the stream of oil in the main channel 2201. The second inlet channel 2203 may introduce an aqueous solution containing a substrate for the enzyme so that aqueous droplets containing molecules of the substrate are also introduced into the main channel 2201. In more detail, droplets containing the enzyme are first sheared off into the main channel 2201 at the first droplet extrusion region 2204. These droplets then move downstream, with the oil stream in the main channel, and pass through the second droplet extrusion region 2205.” Quake at [0316]. Figure 22 from Quake is reproduced below:

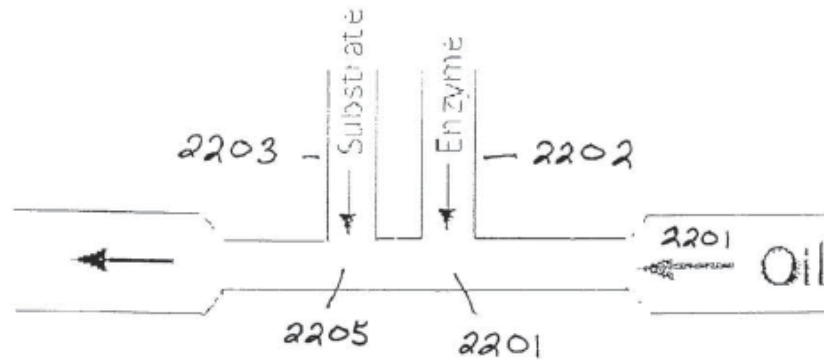


FIG. 22

Quake at Fig. 22. Quake states that “[a]lthough the exemplary embodiment described here, and illustrated in FIG. 22, releases droplets of enzyme upstream from the droplets of substrate, droplets of the different fluid or solutions may be released in any order. Thus, for example, an aqueous solution containing a substrate may be released through the first inlet channel 2202 at the first droplet extrusion region 2204, and droplets of an aqueous solution containing an enzyme may be released through the second inlet channel 2203 at the second droplet extrusion region 2205.” Quake at [0318].

2415. Quake also describes that “in preferred embodiments, the sample inlet and main channel intercept at a T-shaped junction; i.e., such that the sample inlet is perpendicular (90 degrees) to the main channel. However, the sample inlet may intercept the main channel at any angle . . . Other shapes and channel geometries may be used as desired.” Quake at [0084]-[0085]. Indeed, this language was copied almost exactly in Ismagilov’s 60/379,927 provisional application, to which the ’091 patent claims priority. *See* Ismagilov ’927 provisional application at 9:31-39.

2416. It also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would be obvious to

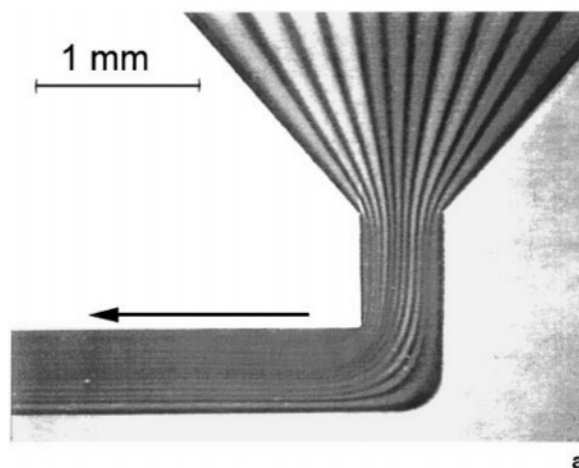
simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2417. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

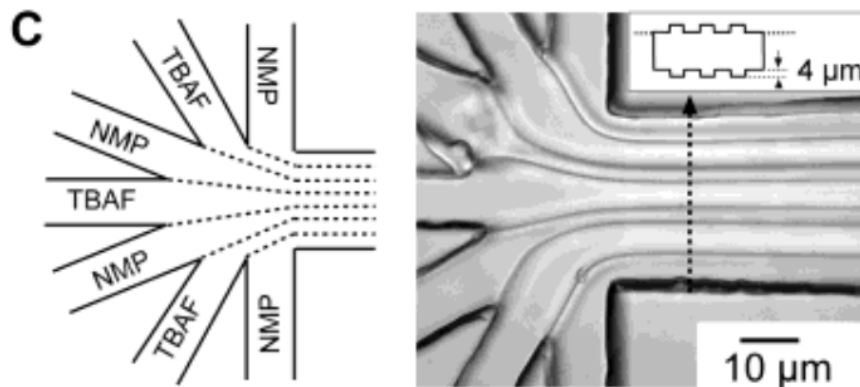
2418. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2419. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids

to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



2420. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

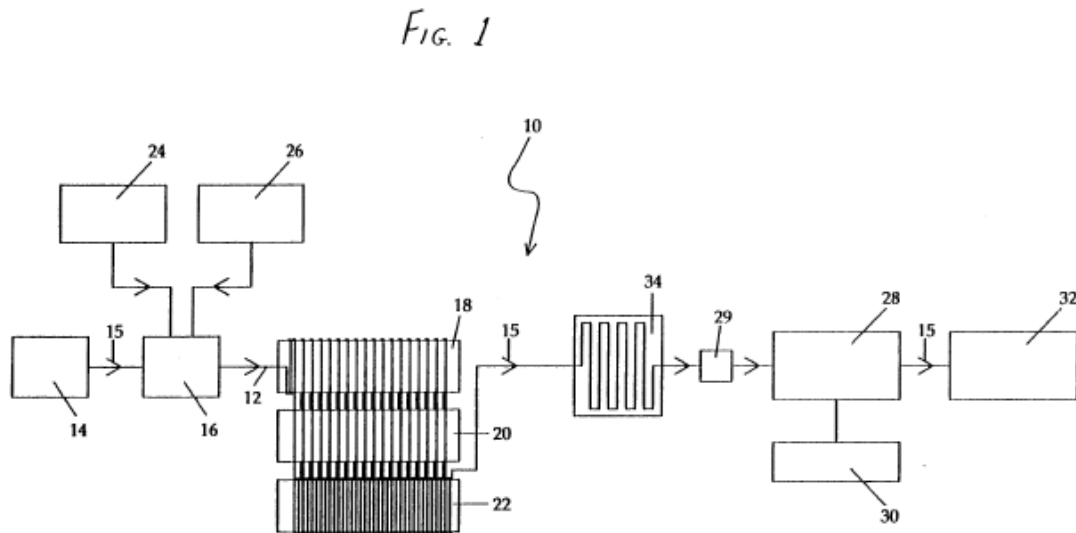


Whitesides at 845-846.

2421. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2422. While it is my opinion that Quake discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse

transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

2423. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v)

hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2424. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2425. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

2426. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2427. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

2428. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

(xiii) *Claim 37*

2429. The preamble of claim 37 of the ’091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2430. I understand that the Court has not considered whether the preamble of this claim is limiting.

2431. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

2432. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

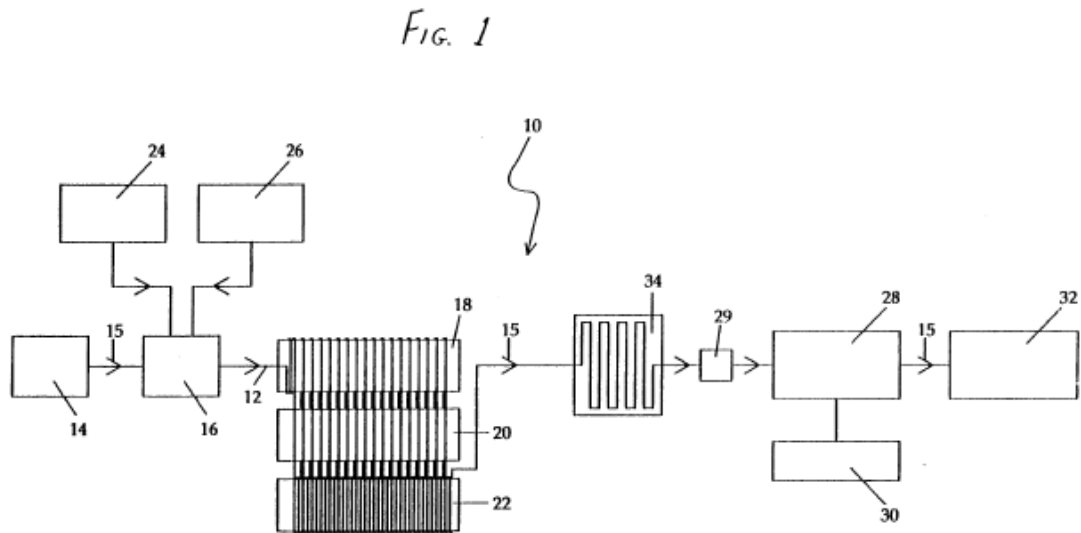
2433. Quake even describes a specific type of chemical reaction involving enzymes produced by cells:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

2434. While it is my opinion that Quake discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction

mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2435. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at

very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2436. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2437. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2438. It also would have been obvious to conduct a reaction within at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2439. Claim 37 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2440. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically

contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2441. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2442. Claim 37 further recites: “**introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2443. Quake at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a *pressurized stream of oil is passed*, and at least one sample inlet channel, through which a *pressurized stream of aqueous solution* is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a pressurized stream or flow of oil in a main channel of the device.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2444. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as *an ‘extrusion’ or ‘barrier’ fluid*, passes (i.e., flows) through the main channel of the device and a *second fluid, referred to as a ‘sample’ or ‘droplet’ fluid*, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main

channel.”).

2445. Quake also describes different droplet-formation configurations. For example, Quake discloses that “[i]n another preferred embodiment, the device of the invention comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a first droplet extrusion region, and a second inlet region in communication with the main channel at a second droplet extrusion region.” Quake at [0018]. Quake also describes that “[i]n the preferred embodiment illustrated in Fig. 22, the first inlet channel 2202 may introduce an aqueous solution containing an enzyme so that aqueous droplets containing molecules of the enzyme are introduced into the stream of oil in the main channel 2201. The second inlet channel 2203 may introduce an aqueous solution containing a substrate for the enzyme so that aqueous droplets containing molecules of the substrate are also introduced into the main channel 2201. In more detail, droplets containing the enzyme are first sheared off into the main channel 2201 at the first droplet extrusion region 2204. These droplets then move downstream, with the oil stream in the main channel, and pass through the second droplet extrusion region 2205.” Quake at [0316]. Figure 22 from Quake is reproduced below:

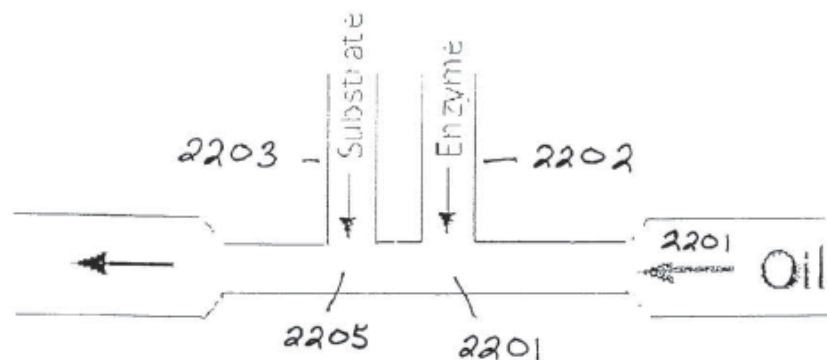


FIG. 22

Quake at Fig. 22. Quake states that “[a]lthough the exemplary embodiment described here, and illustrated in FIG. 22, releases droplets of enzyme upstream from the droplets of substrate, droplets of the different fluid or solutions may be released in any order. Thus, for example, an aqueous solution containing a substrate may be released through the first inlet channel 2202 at the first droplet extrusion region 2204, and droplets of an aqueous solution containing an enzyme may be released through the second inlet channel 2203 at the second droplet extrusion region 2205.” Quake at [0318].

2446. Quake also describes that “in preferred embodiments, the sample inlet and main channel intercept at a T-shaped junction; i.e., such that the sample inlet is perpendicular (90 degrees) to the main channel. However, the sample inlet may intercept the main channel at any angle . . . Other shapes and channel geometries may be used as desired.” Quake at [0084]-[0085]. Indeed, this language was copied almost exactly in Ismagilov’s 60/379,927 provisional application, to which the ’091 patent claims priority. *See* Ismagilov ’927 provisional application at 9:31-39.

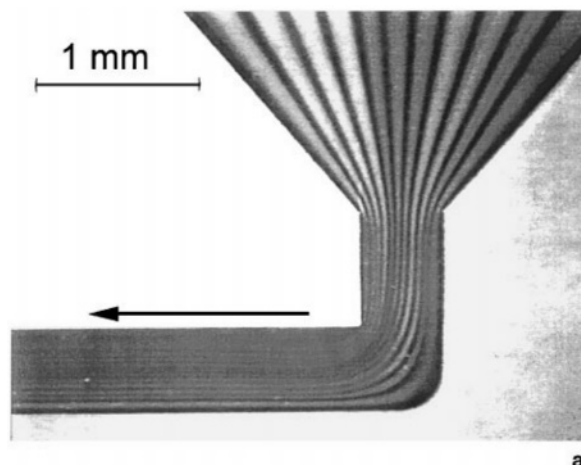
2447. It also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2448. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a

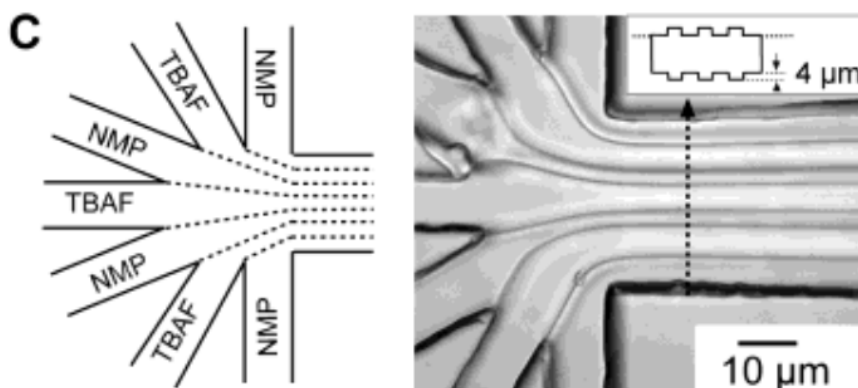
microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2449. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2450. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2451. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

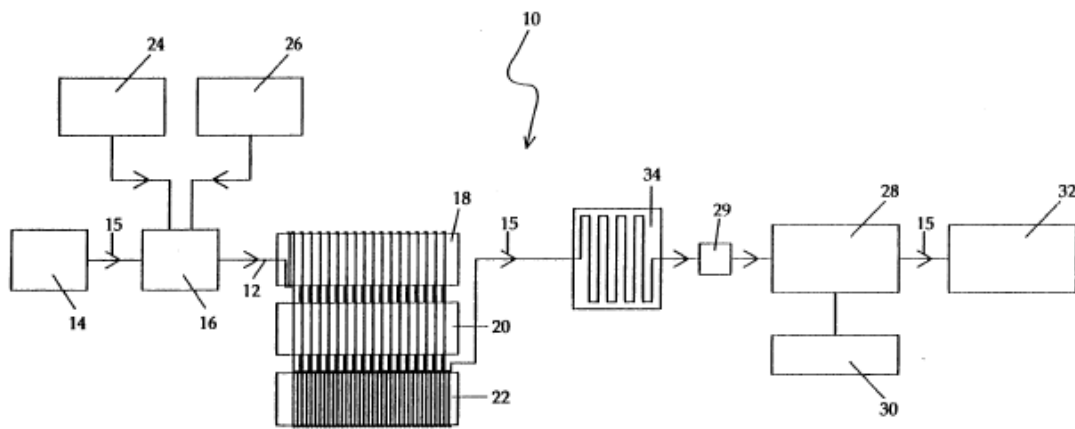


Whitesides at 845-846.

2452. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2453. While it is my opinion that Quake discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2454. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2455. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2456. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2457. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2458. Claim 37 further recites: “**each plug is substantially surrounded by carrier.**”

2459. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

(xiv) *Claim 38*

2460. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2461. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

2462. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within *microfluidic channels filled with oil.* The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at

least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0014] (“In a preferred embodiment, water droplets are extruded into a flow of oil”); Quake at [0015] (“For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).”); Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane or hexadecane)”; Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane)”).

2463. Quake also explains that the carrier fluid, or “extrusion fluid,” is “incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel.” Quake at [0022].

2464. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

(xv) *Claim 39*

2465. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2466. Claim 39 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2467. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or

frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

2468. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel . . . The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

2469. Quake also describes that the surfactant can coat the microchannel walls. For example, Quake describes that “[t]o prevent material (e.g., cells, virions and other particles or molecules) from adhering to the sides of the channels, the channels . . . may have a coating which minimizes adhesion Alternatively, the channels may be coated with a surfactant.” Quake at [0094]; *see also* Quake at [0118] (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.”).

2470. Quake further described experimental testing using oils containing surfactants. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices,

including decane, tetradecane and hexadecane. In each instance, the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol./vol.) of either 0.5, 1.0 or 2.0%.” Quake at [0300].

(xvi) *Claim 43*

2471. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2472. Claim 43 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

2473. Quake satisfies this limitation, describing a specific kind of reaction with a soluble reaction product:

In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

(xvii) *Claim 53*

2474. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2475. Claim 53 further recites: “**employing a number of devices in parallel.**”

2476. I understand that Bio-Rad is contending that “microfluidic chips with eight channels in parallel” fall within the scope of this claim. *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 165. Under Plaintiffs’ interpretation of the term, Quake satisfies this limitation. For example, Quake states that “[a]n ‘analysis unit’ is a microfabricated substrate, e.g., a micro fabricated chip, having at least one inlet region, at least one main channel, at least one detection region and at least one outlet region . . . ***A device according to the invention may comprise a plurality of analysis units.***” Quake at [0067].

2477. It also would have been obvious employ a number of devices in parallel based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) *Claim 56*

2478. Claim 56 of the ’091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2479. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

2480. Quake satisfies this limitation. For example, Quake discloses that “[i]n preferred embodiments, the droplets of aqueous solution have a volume of approximately 0.1 to 100 picoliters (pL).” Quake at [0003].

2481. Quake also explains that “[f]or particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are preferably rounded, with a diameter between 2

and 100 microns, preferably about 60 microns, and more preferably about 30 microns at the crossflow area or droplet extrusion region . . . Similarly, the volume of the detection region in an analysis device is typically in the range of between about 10 femtoliters (fl) and 5000 fl, preferably about 40 or 50 fl to about 1000 or 2000 fl, most preferably on the order of about 200 fl. In preferred embodiments, the channels of the device, and particularly the channels of the inlet connecting to a droplet extrusion region, are between about 2 and 50 microns, most preferably about 30 microns.” Quake at [0091].

2482. Quake also provides a formula that can be used to calculate droplet size:

The size of a droplet in a micro fluidic device of this invention may be provided by the equation:

$$r = \frac{\sigma}{\eta \varepsilon}$$

where r is the final droplet radius in a main channel. η , the viscosity of the continuous phase (e.g., the oil-surfactant phase in the above exemplary devices) and σ , the interfacial tension, may be obtained from values available in the art for the particular fluids used (see, for example, CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Fla., 2000). ε , which denotes the shear rate, may be provided by the formula

$$\varepsilon = \frac{2}{Y_0} v,$$

where v is the velocity of the dispersed phase fluid (i.e., the droplets) and may be readily calibrated to the input pressures for a particular microfluidic device. Y_0 denotes the radius of the inlet channel at the droplet extrusion region (i.e., the radius of the tapered channel 1606 in FIG. 16B).

Quake at [0308]-[0310].

(xix) Claim 57

2483. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2484. I understand that the Court has not considered whether the preamble of this claim is limiting.

2485. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

2486. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

2487. Quake even describes a specific type of chemical reaction involving enzymes produced by cells:

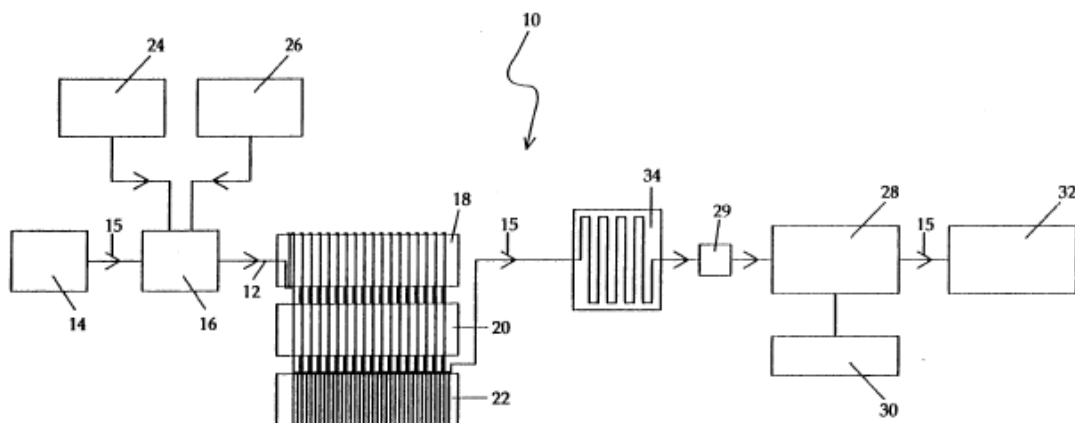
In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability

of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

2488. While it is my opinion that Quake discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2489. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2490. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2491. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2492. It also would have been obvious to conduct a reaction within at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2493. Claim 57 further recites: “**introducing a carrier-fluid into a first microchannel**

of a device.”

2494. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

2495. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2496. Claim 57 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

2497. Quake at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a *pressurized stream of oil is passed*, and at least one sample inlet channel, through which a *pressurized stream of aqueous solution* is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a pressurized stream or flow of oil in a main channel of the device.”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an

angle perpendicular to the flow of fluid in the main channel.”).

2498. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a *second fluid, referred to as a ‘sample’ or ‘droplet’ fluid*, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.”).

2499. Quake also describes different droplet-formation configurations. For example, Quake discloses that “[i]n another preferred embodiment, the device of the invention comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a first droplet extrusion region, and a second inlet region in communication with the main channel at a second droplet extrusion region.” Quake at [0018]. Quake also describes that “[i]n the preferred embodiment illustrated in Fig. 22, the first inlet channel 2202 may introduce an aqueous solution containing an enzyme so that aqueous droplets containing molecules of the enzyme are introduced into the stream of oil in the main channel 2201. The second inlet channel 2203 may introduce an aqueous solution containing a substrate for the enzyme so that aqueous droplets containing molecules of the substrate are also introduced into the main channel 2201. In more detail, droplets containing the enzyme are first sheared off into the main channel 2201 at the first droplet extrusion region 2204. These droplets then move downstream, with the oil stream in the main channel, and pass through the second droplet extrusion region 2205.” Quake at [0316]. Figure 22 from Quake is reproduced below:

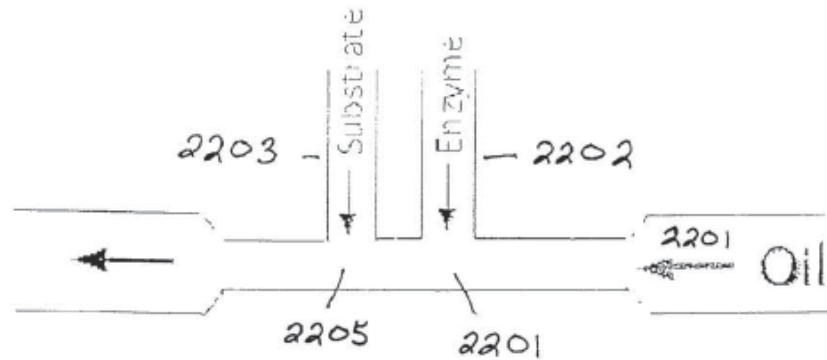


FIG. 22

Quake at Fig. 22. Quake states that “[a]lthough the exemplary embodiment described here, and illustrated in FIG. 22, releases droplets of enzyme upstream from the droplets of substrate, droplets of the different fluid or solutions may be released in any order. Thus, for example, an aqueous solution containing a substrate may be released through the first inlet channel 2202 at the first droplet extrusion region 2204, and droplets of an aqueous solution containing an enzyme may be released through the second inlet channel 2203 at the second droplet extrusion region 2205.” Quake at [0318].

2500. Quake also describes that “in preferred embodiments, the sample inlet and main channel intercept at a T-shaped junction; i.e., such that the sample inlet is perpendicular (90 degrees) to the main channel. However, the sample inlet may intercept the main channel at any angle . . . Other shapes and channel geometries may be used as desired.” Quake at [0084]-[0085]. Indeed, this language was copied almost exactly in Ismagilov’s 60/379,927 provisional application, to which the ’091 patent claims priority. *See* Ismagilov ’927 provisional application at 9:31-39.

2501. It also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would be obvious to

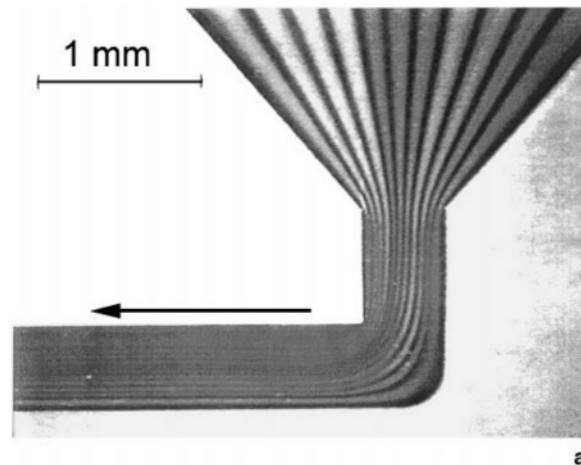
simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2502. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

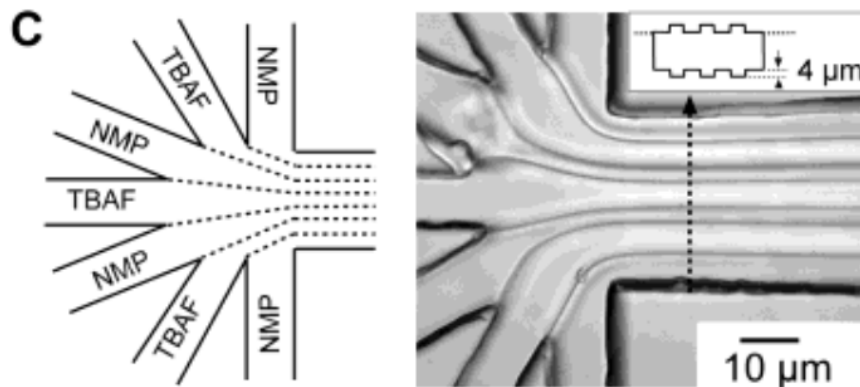
2503. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2504. It also would be obvious to simultaneously introduce two streams of plug-

fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



2505. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

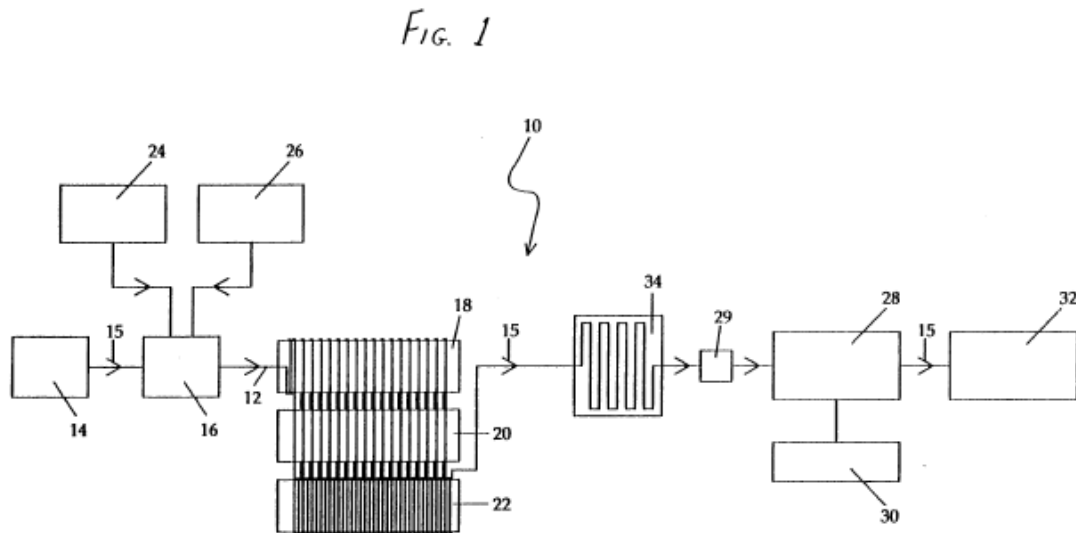


Whitesides at 845-846.

2506. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2507. While it is my opinion that Quake discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse

transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μl , is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2508. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2509. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2510. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for

producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2511. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2512. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

2513. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

(xx) *Claim 58*

2514. Claim 58 of the ’091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

2515. Claim 58 further recites: “**each plug initially has a cross section that is**

substantially the same size as the cross section of the channel at the junction area.”

2516. Quake satisfies this limitation. For example, Quake discloses that “[i]n one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes.” Quake at [0092].

2517. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(b) Invalidity Based on Shaw Stewart

2518. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the ’091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

2519. The preamble of claim 1 of the ’091 patent recites: “**A method of conducting a reaction within at least one plug.**”

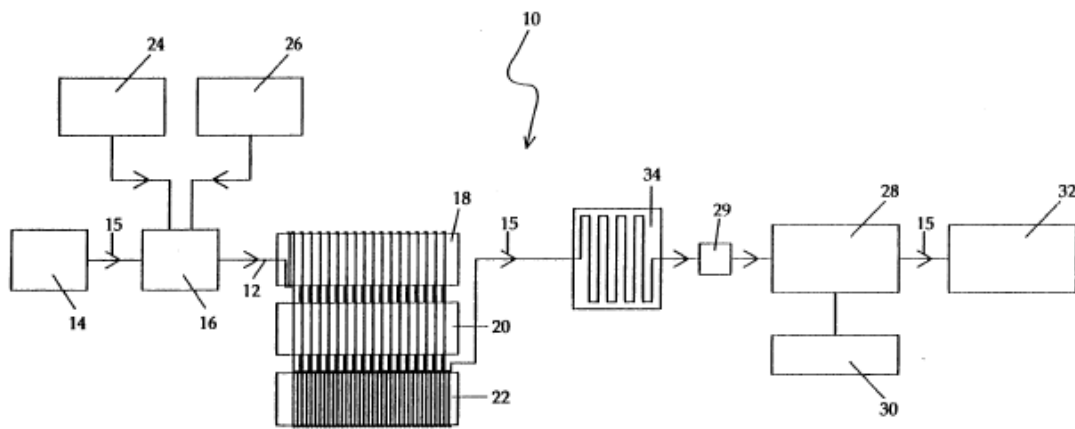
2520. I understand that the Court has not considered whether the preamble of this claim is limiting.

2521. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described

was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

2522. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2523. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2524. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2525. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that reactions could be conducted within droplets in a microfluidic system.

2526. It also would have been obvious to conduct a reaction within at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2527. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2528. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

2529. Figure 1 of Shaw Stewart also discloses this limitation.

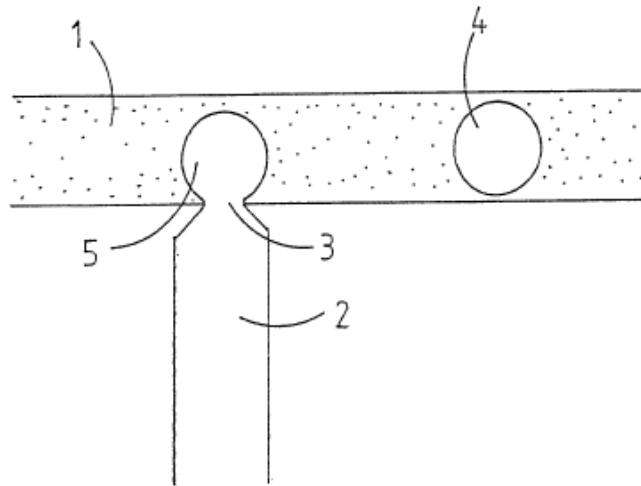


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2530. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2531. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while *a continuous current of carrier phase*

flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

2532. Figure 1 of Shaw Stewart also discloses this limitation.

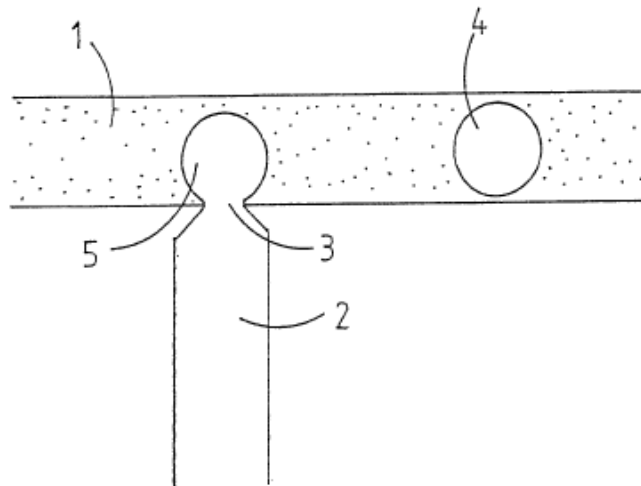


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2533. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw

Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

2534. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with Quake. I incorporate my analysis with respect to ¶¶ 2332-2335, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

2535. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet

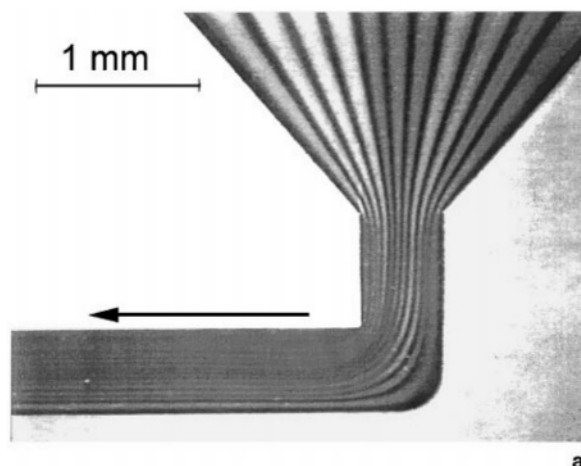
in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2536. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

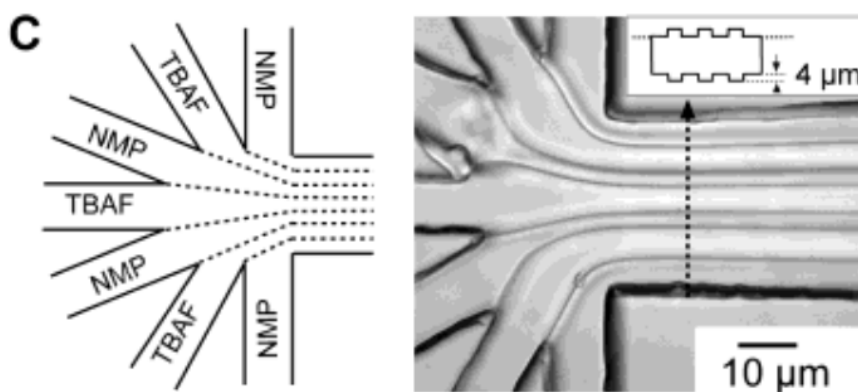
2537. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2538. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is

reproduced below:



2539. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

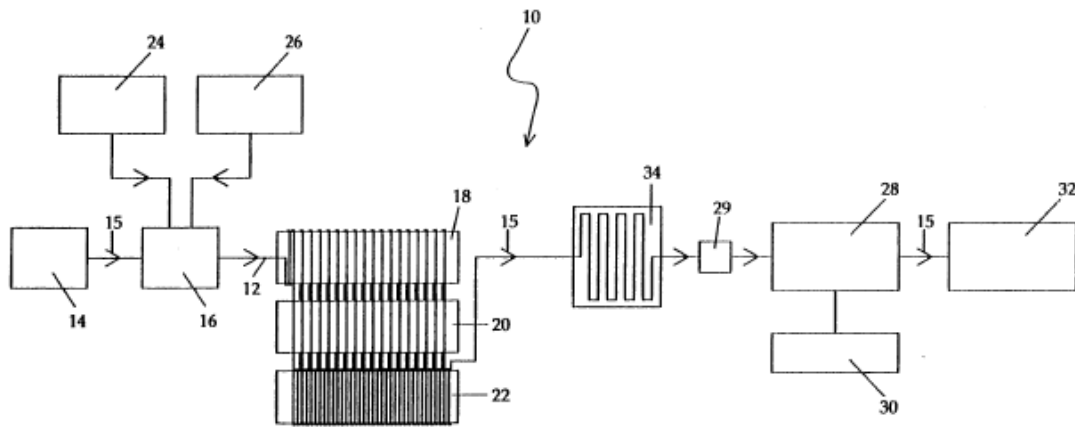


Whitesides at 845-846.

2540. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2541. While it is my opinion that Shaw Stewart discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2542. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2543. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2544. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

2545. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Shaw Stewart in light of the background

knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2546. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

2547. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

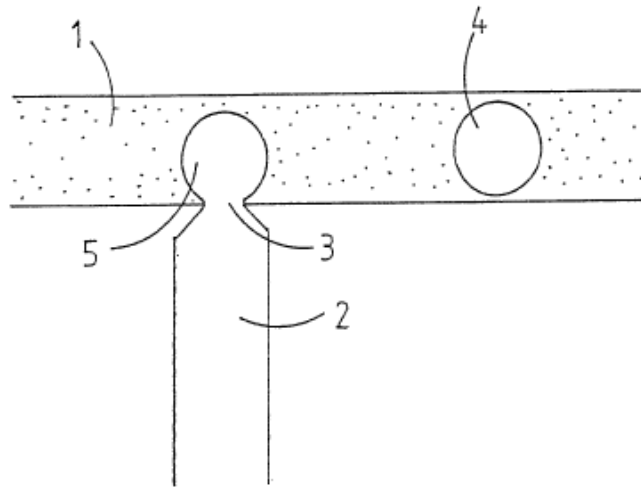


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2548. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

(ii) *Claim 2*

2549. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my

analysis with respect to claim 1.

2550. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

2551. Shaw Stewart satisfies this limitation. Shaw Stewart disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

(iii) *Claim 3*

2552. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2553. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

2554. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.” Shaw Stewart II at 4 (emphasis added).

2555. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2556. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing

agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2557. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2558. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

2559. Claim 5 of the ’091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2560. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2561. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in

the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxyol, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(v) *Claim 6*

2562. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2563. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

2564. Shaw Stewart satisfies this limitation. Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66.

(vi) *Claim 11*

2565. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2566. Claim 11 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

2567. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart

also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended biological micro-organisms.” Shaw Stewart at 4:30-33.

(vii) *Claim 27*

2568. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2569. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

2570. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

2571. A POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* '091 Patent at Table 1 (refractive index of water is 1.3330) *to* Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

(viii) *Claim 29*

2572. Claim 29 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2573. Claim 29 further recites: “**employing a number of devices in parallel.**”

2574. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2378-2380, demonstrating how Quake discloses employing a number of devices in parallel.

2575. It also would have been obvious to employ a number of devices in parallel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 31*

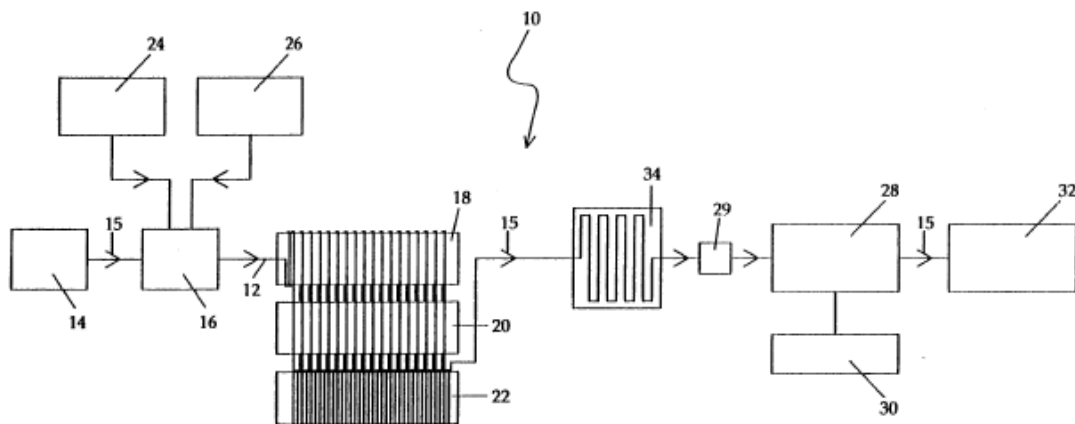
2576. Claim 31 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2577. Claim 31 further recites: “**the reaction is a polymerization reaction.**”

2578. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart

with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2579. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2580. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2581. It also would have been obvious to conduct a polymerization reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

2582. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2583. Claim 33 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.”**

2584. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2389-2391, demonstrating how Quake discloses that each plug initially has a cross section that is substantially the same size as the cross section of the channel

at the inlet.

2585. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

2586. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2587. Claim 35 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

2588. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2393-2397, demonstrating how Quake discloses that the volume of at least one plug is about 1 femtoliter to about 250nL.

2589. It also would have been obvious to have the volume of at least one plug about 1 femtoliter to about 250nL based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 36*

2590. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a**

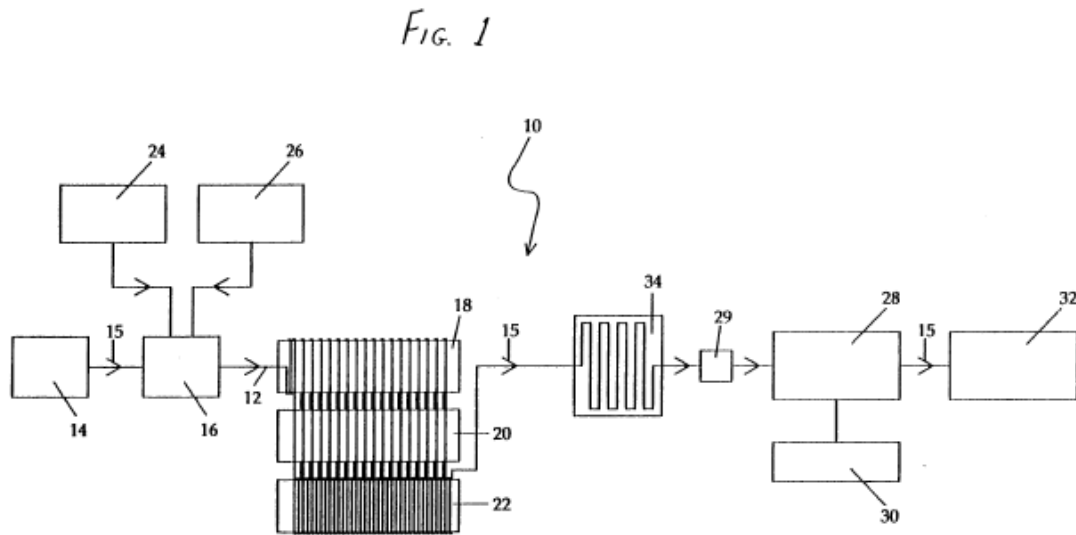
reaction within at least one plug.”

2591. I understand that the Court has not considered whether the preamble of this claim is limiting.

2592. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

2593. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is

reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2594. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a

passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2595. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2596. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are

“micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

2597. It also would have been obvious to conduct a reaction within at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2598. Claim 36 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2599. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

2600. Figure 1 of Shaw Stewart also discloses this limitation.

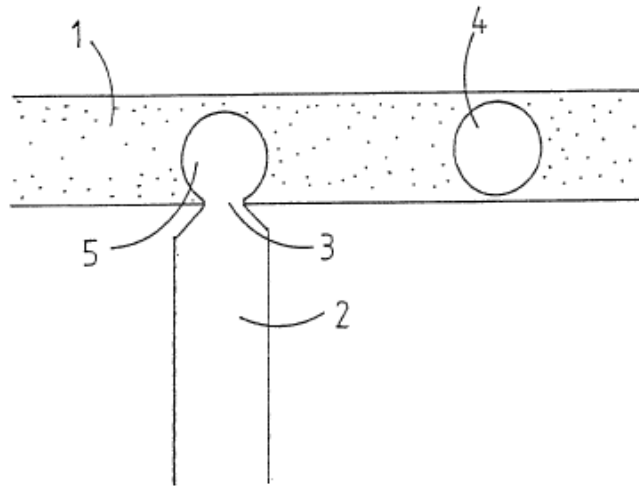


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2601. Claim 36 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2602. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a *continuous current of carrier phase*

flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

2603. Figure 1 of Shaw Stewart also discloses this limitation.

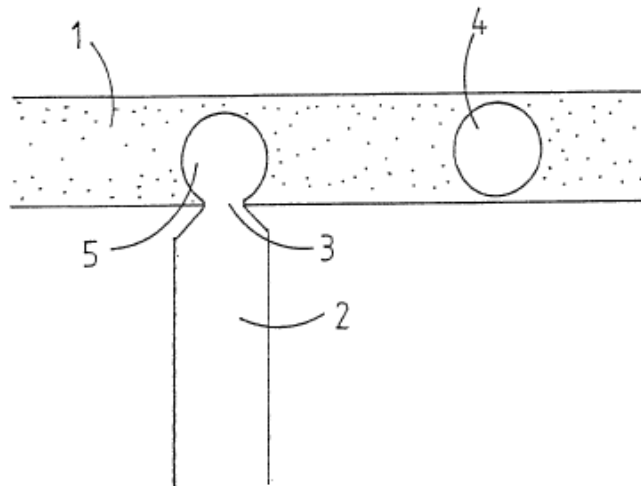


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2604. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw

Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

2605. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with Quake. I incorporate my analysis with respect to ¶¶ 2412-2415, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

2606. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet

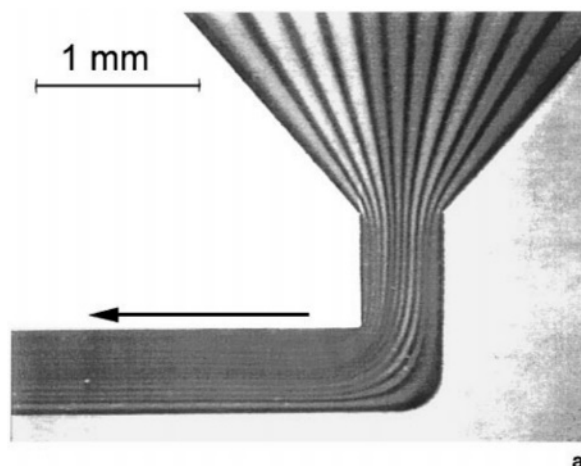
in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2607. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

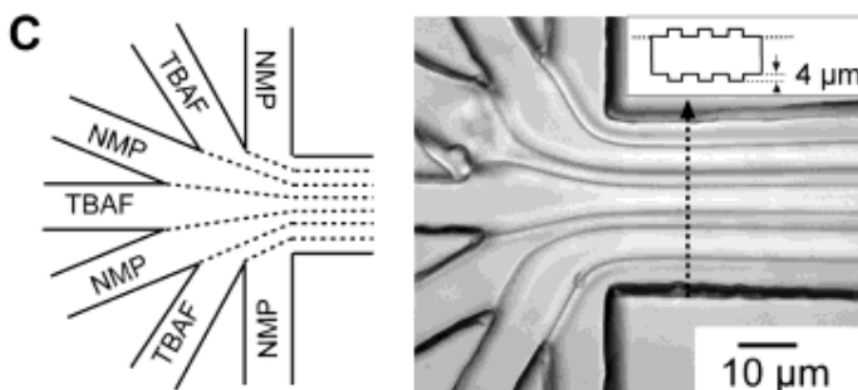
2608. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2609. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is

reproduced below:



2610. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

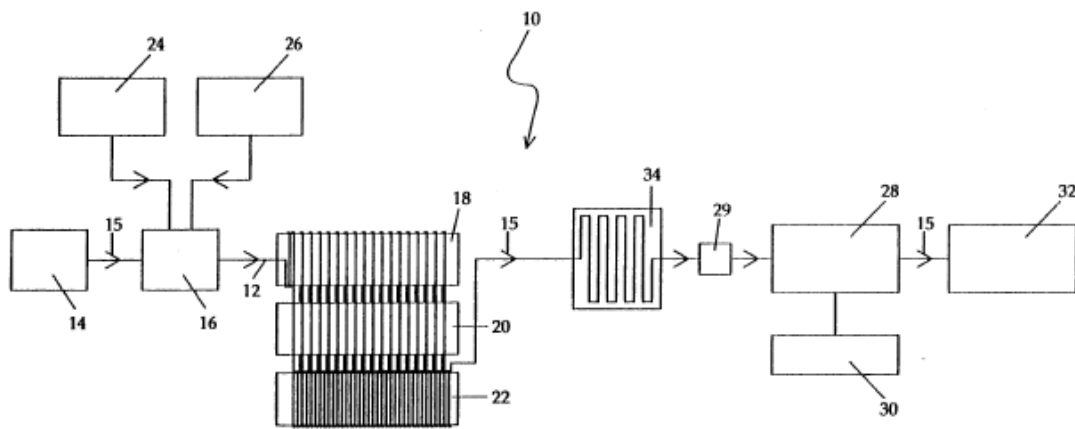


Whitesides at 845-846.

2611. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2612. While it is my opinion that Shaw Stewart discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2613. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2614. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2615. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2616. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2617. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

2618. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

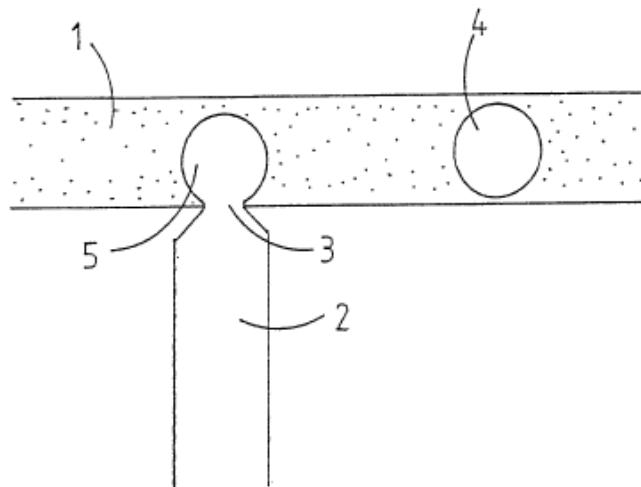


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2619. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

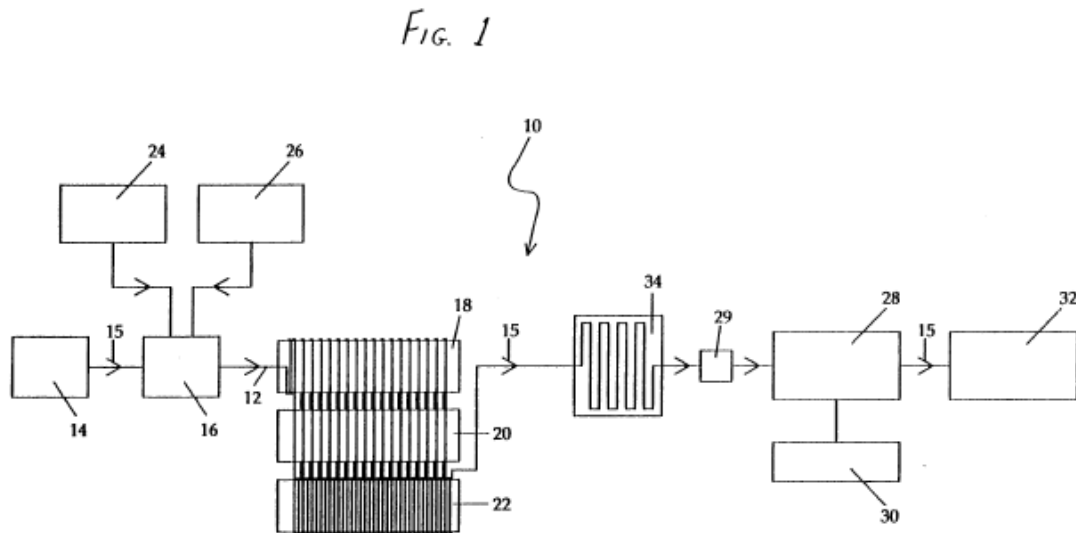
(xiii) *Claim 37*

2620. The preamble of claim 37 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2621. I understand that the Court has not considered whether the preamble of this claim is limiting. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter . . .” Shaw Stewart at 1:20-22 (emphasis added).

2622. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse

transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μl , is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2623. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a

syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2624. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2625. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30)

together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2626. It also would have been obvious to conduct a reaction within at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2627. Claim 37 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2628. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include

mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

2629. Figure 1 of Shaw Stewart also discloses this limitation.

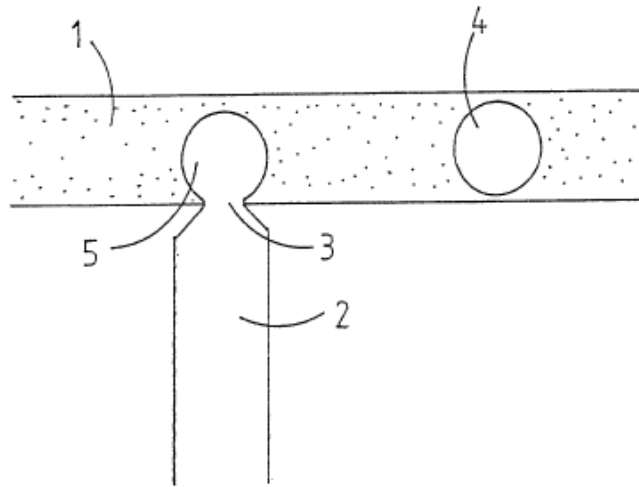


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2630. Claim 37 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

2631. Shaw Stewart at least renders obvious this limitation, in light of the background

knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a *continuous current of carrier phase* flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

2632. Figure 1 of Shaw Stewart also discloses this limitation.

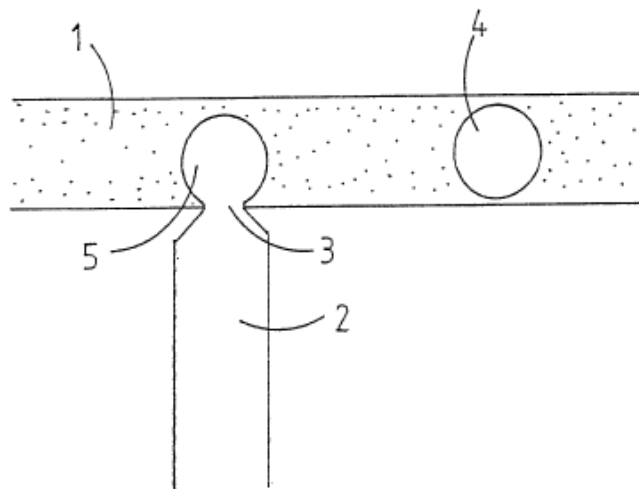


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small

opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2633. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

2634. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with Quake. I incorporate my analysis with respect to ¶¶ 2443-2446, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

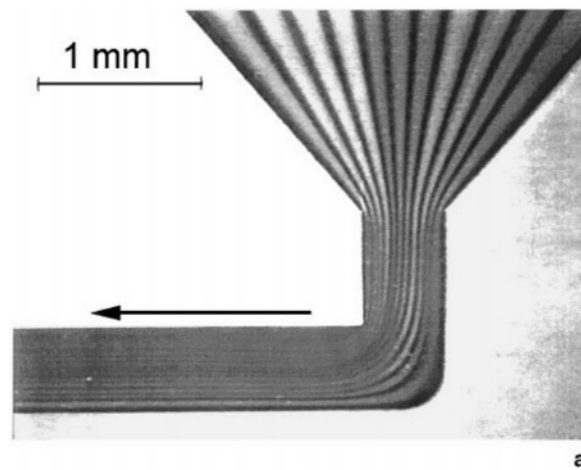
2635. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2636. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

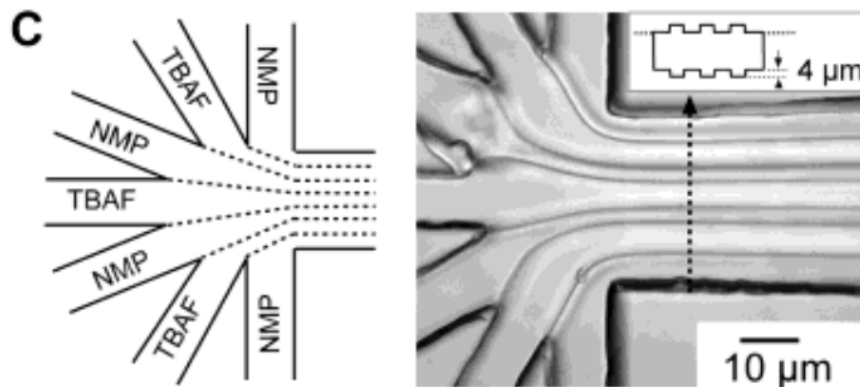
2637. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-

16.

2638. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



2639. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



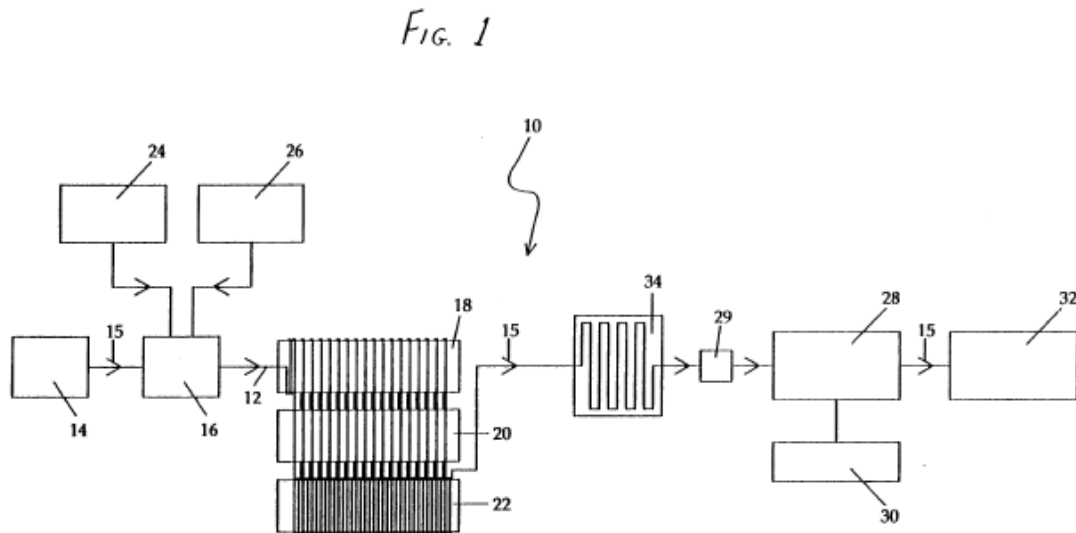
Whitesides at 845-846.

2640. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2641. While it is my opinion that Shaw Stewart discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative

thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2642. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2643. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2644. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the

carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

2645. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2646. Claim 37 further recites: “**each plug is substantially surrounded by carrier.**”

2647. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

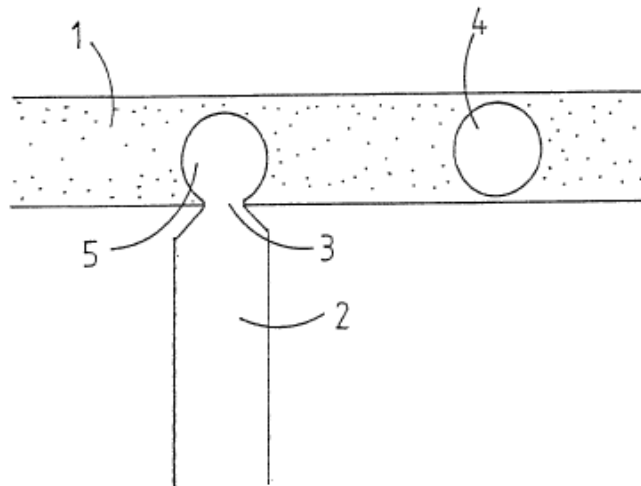


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2648. Shaw Stewart also states that the method it claims involves “discrete volumes of

chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

(xiv) *Claim 38*

2649. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2650. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

2651. Shaw Stewart satisfies this limitation. Shaw Stewart disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

(xv) *Claim 39*

2652. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2653. Claim 39 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2654. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]*urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.*” Suitable carrier phases include cholesterol, sodium dioxyol, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

(xvi) *Claim 43*

2655. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2656. Claim 43 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

2657. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended biological micro-organisms.” Shaw Stewart at 4:30-33.

(xvii) *Claim 53*

2658. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2659. Claim 53 further recites: “**employing a number of devices in parallel.**”

2660. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2474-2477, demonstrating how Quake discloses employing a number of devices in parallel.

2661. It also would have been obvious employ a number of devices in parallel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections

VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) Claim 56

2662. Claim 56 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2663. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

2664. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2478-2482, demonstrating how Quake discloses that the volume of at least one plug is about 1 femtoliter to about 250nL.

2665. It also would have been obvious to have a volume of at least one plug about 1 femtoliter to about 250nL based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xix) Claim 57

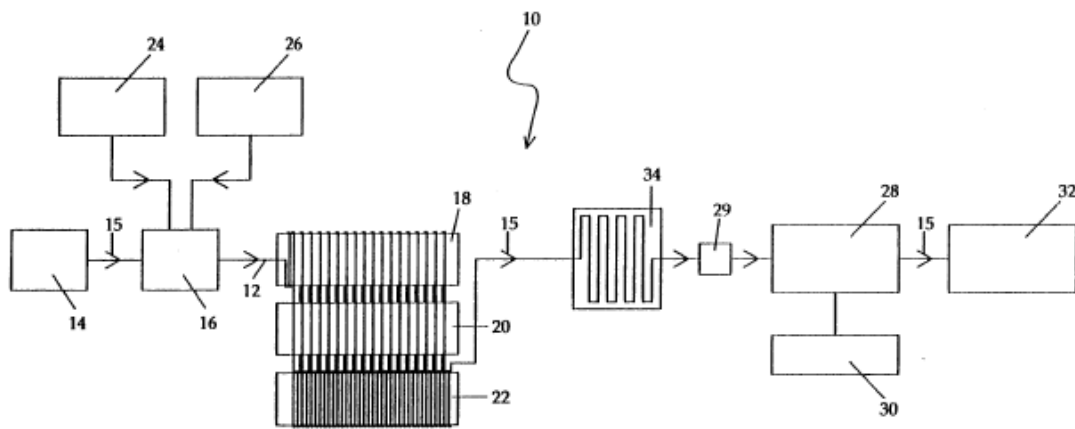
2666. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2667. I understand that the Court has not considered whether the preamble of this claim is limiting.

2668. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

2669. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2670. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2671. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2672. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that reactions could be conducted within droplets in a microfluidic system.

2673. It also would have been obvious to conduct a reaction within at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2674. Claim 57 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2675. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

2676. Figure 1 of Shaw Stewart also discloses this limitation.

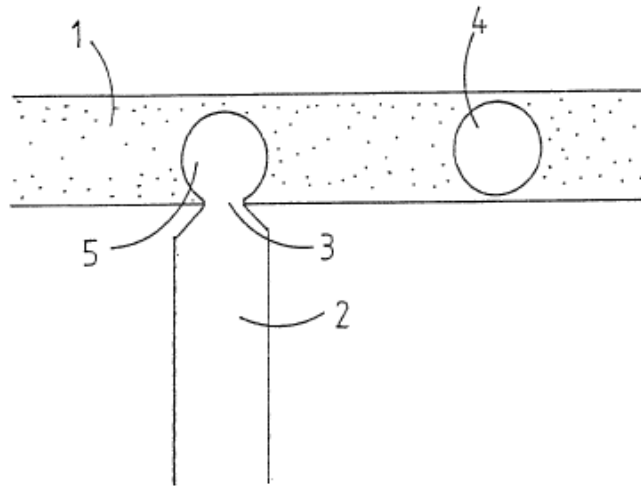


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2677. Claim 57 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

2678. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of*

reagent through the opening will be produced, while a *continuous current of carrier phase* flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that this continuous flow of aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33.

2679. Figure 1 of Shaw Stewart also discloses this limitation.

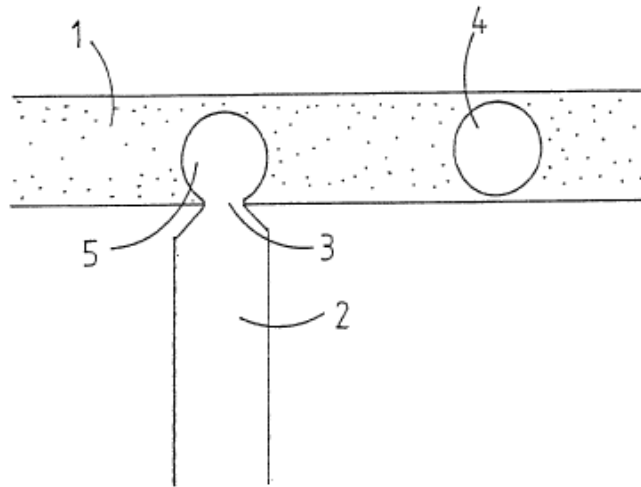


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2680. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

2681. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with Quake. I incorporate my analysis with respect to ¶¶ 2497-2500, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

2682. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would be obvious to

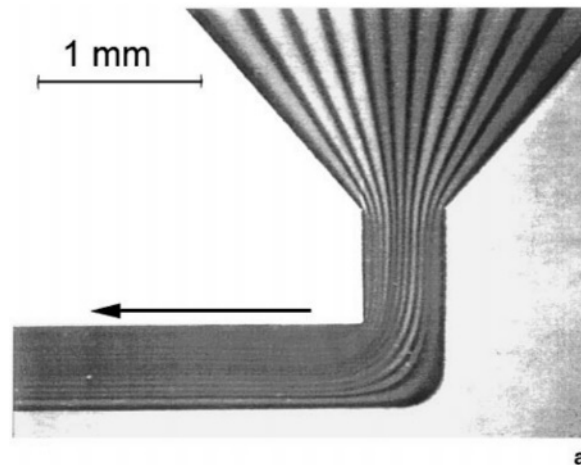
simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2683. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

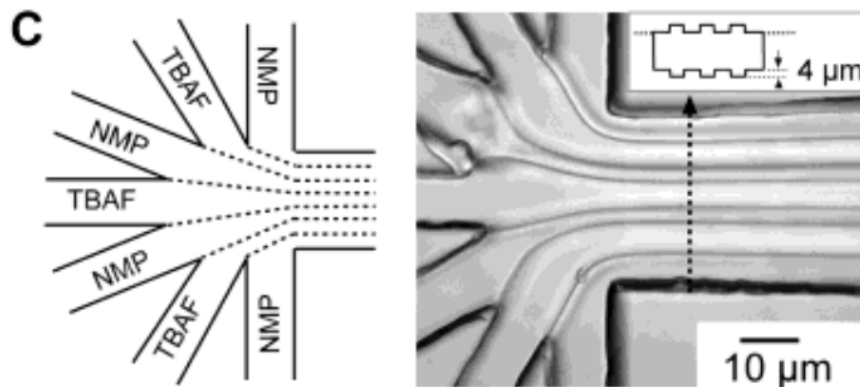
2684. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2685. It also would be obvious to simultaneously introduce two streams of plug-

fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



2686. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



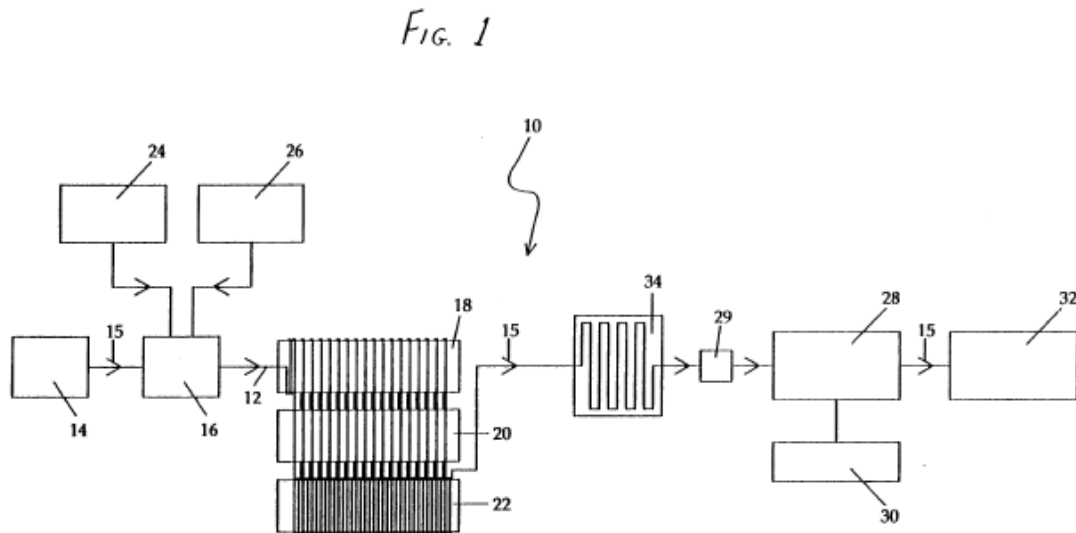
Whitesides at 845-846.

2687. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2688. While it is my opinion that Shaw Stewart discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative

thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2689. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

2690. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

2691. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the

carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

2692. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2693. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

2694. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart also discloses this limitation.

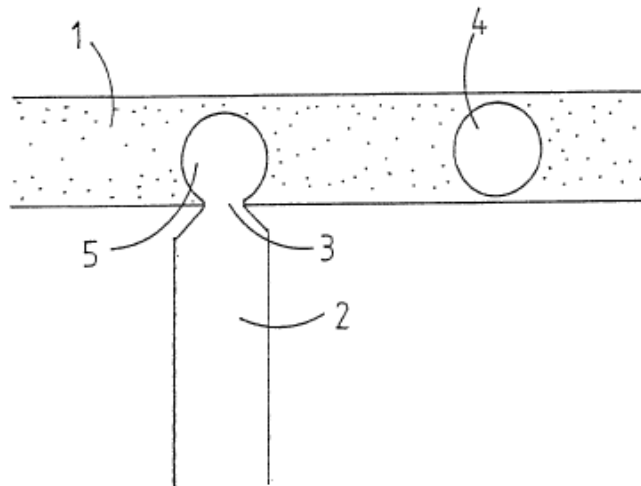


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

2695. Shaw Stewart also states that the method it claims involves “discrete volumes of

chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

(xx) *Claim 58*

2696. Claim 58 of the '091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

2697. Claim 58 further recites: “**each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area.**”

2698. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2514-2516, demonstrating how Quake discloses that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.

2699. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(c) Invalidity Based on Burns (2001)

2700. It is my opinion that Burns (2001) discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the

claim(s) upon which they depend.

(i) *Claim 1*

2701. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2702. I understand that the Court has not considered whether the preamble of this claim is limiting.

2703. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

2704. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2705. This reaction is illustrated in Figure 4:

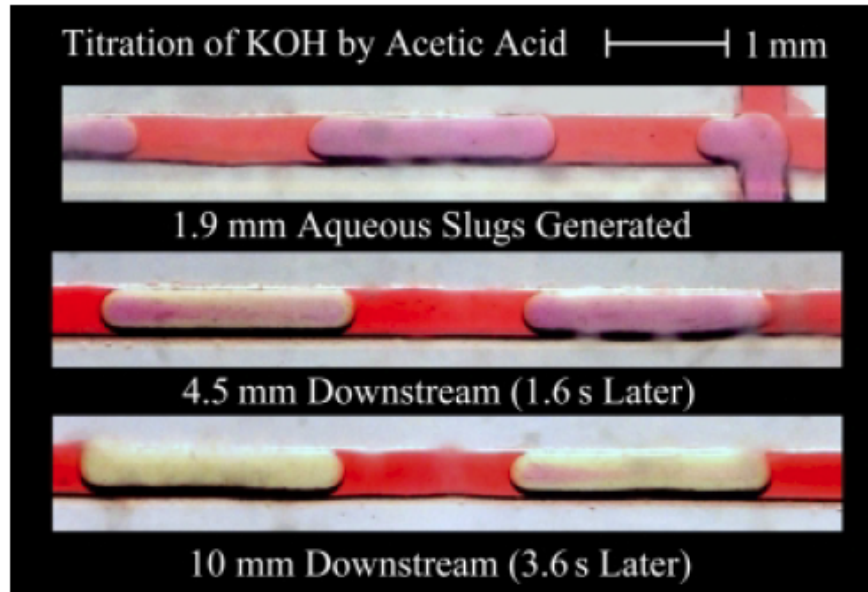
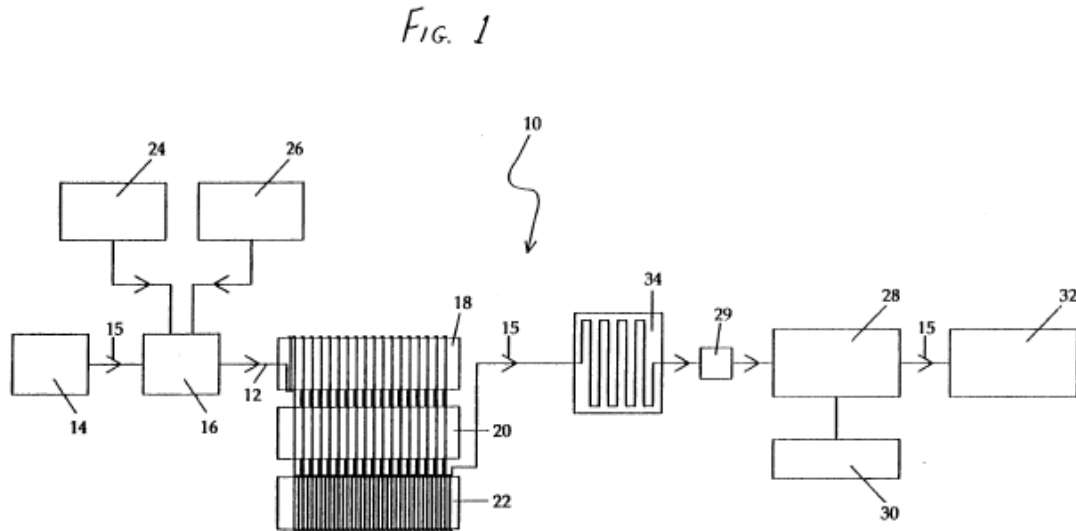


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2706. While it is my opinion that Burns (2001) discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into

tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2707. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from

the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2708. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2709. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may

react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

2710. It also would have been obvious to conduct a reaction within at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2711. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2712. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2713. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a

saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2714. This reaction is illustrated in Figure 4:

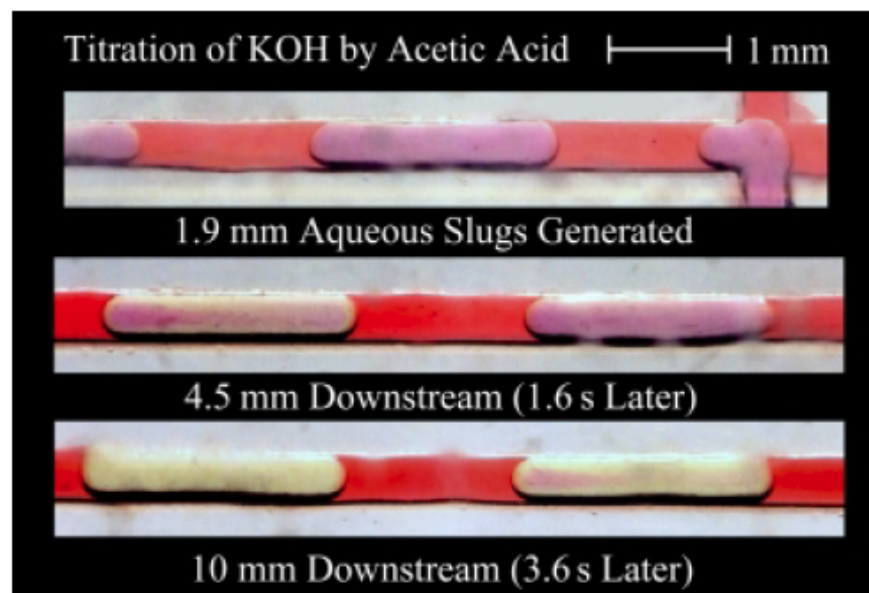


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2715. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in**

the plug.”

2716. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2717. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2718. This reaction is illustrated in Figure 4:

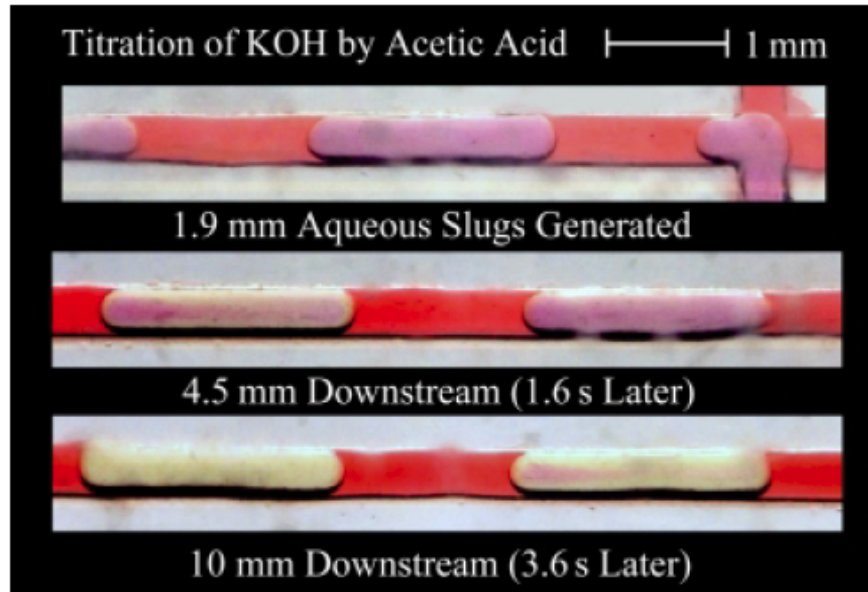


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2719. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with Quake. I incorporate my analysis with respect to ¶¶ 2332-2335, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

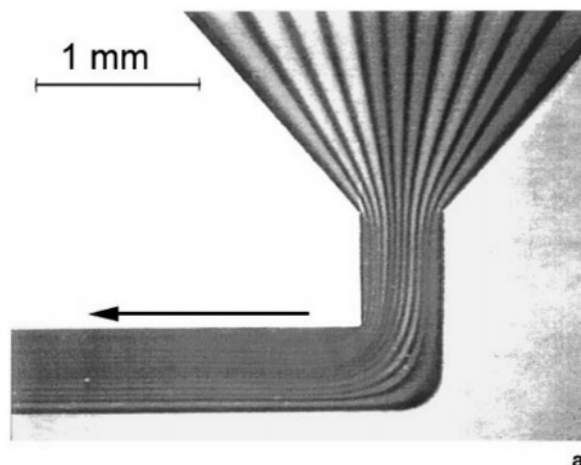
2720. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2721. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in

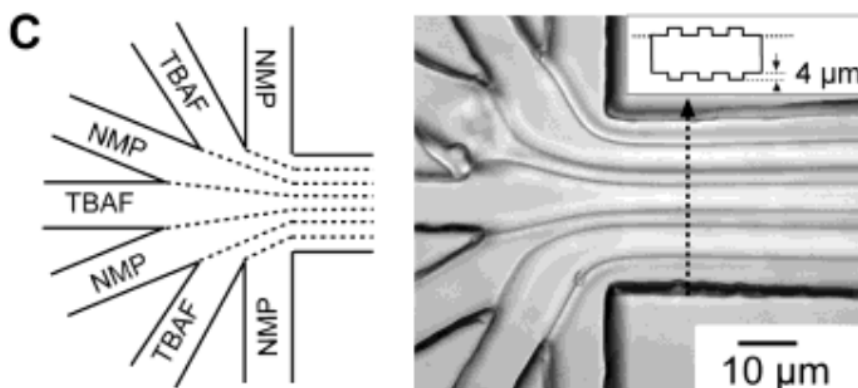
view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2722. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2723. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2724. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

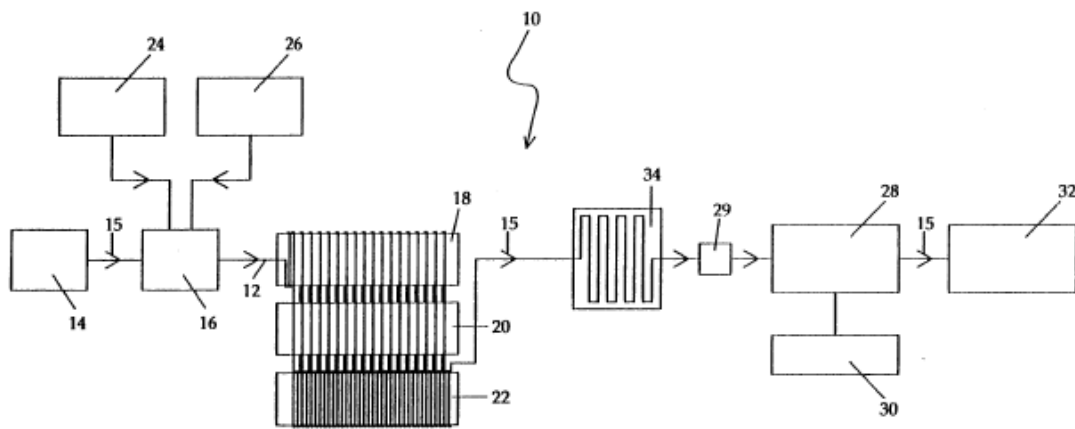


Whitesides at 845-846.

2725. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2726. While it is my opinion that Burns (2001) discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2727. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2728. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2729. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2730. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2731. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

2732. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

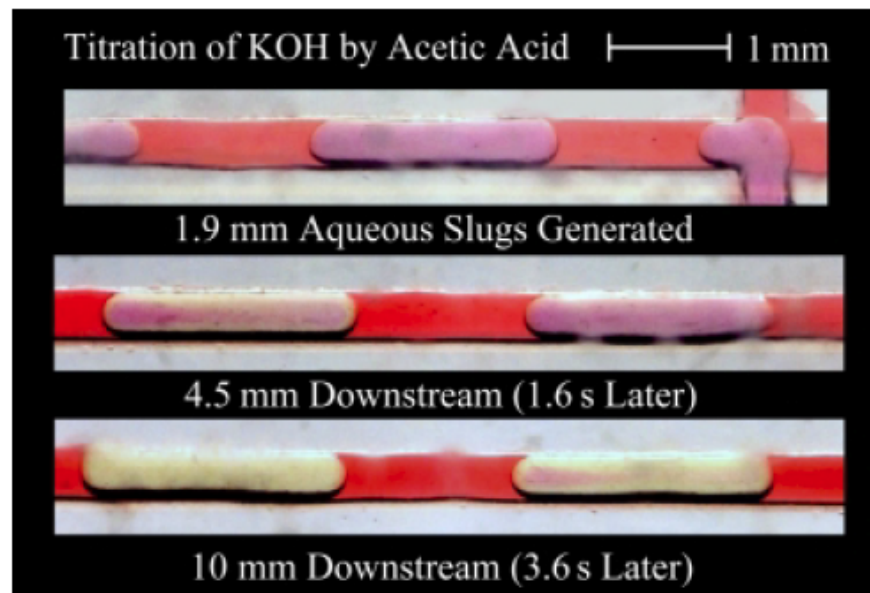


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2733. When a less viscous fluid moves as a “plug” through a more viscous fluid in a

micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

(ii) *Claim 2*

2734. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2735. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

2736. Burns (2001) satisfies this limitation. For example, Burns (2001) makes clear that the carrier fluid is an oil. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. ***Kerosene was used as the basis of the organic phase*** with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene

to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

(iii) *Claim 3*

2737. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2738. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

2739. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2740. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2741. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid

are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2742. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

2743. Claim 5 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2744. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2745. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2746. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft,

at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2747. It also would have been obvious to use a carrier-fluid comprising a surfactant view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2748. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 6*

2749. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2750. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

2751. Burns (2001) satisfies this limitation. For example, Burns (2001) describes an acid-base reaction in which “aqueous solutions of KOH and NaOH” were used. Burns (2001) at

11.

2752. Figure 4 also makes clear that an aqueous phase is used to create droplets:

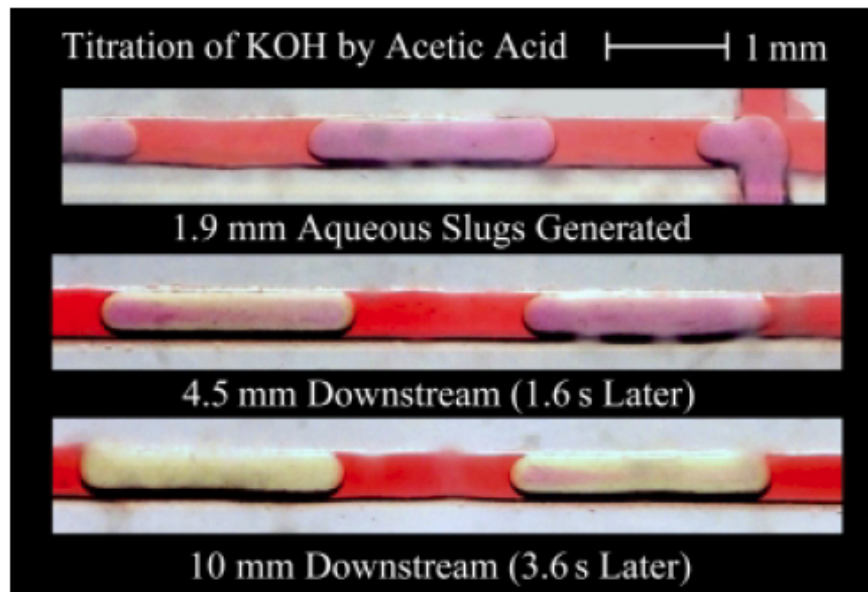


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

(vi) *Claim 11*

2753. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2754. Claim 11 further recites: **“the reaction of the plug-fluids forms a soluble reaction product within at least one plug.”**

2755. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses that “[a] simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was

added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2756. This reaction is illustrated in Figure 4:

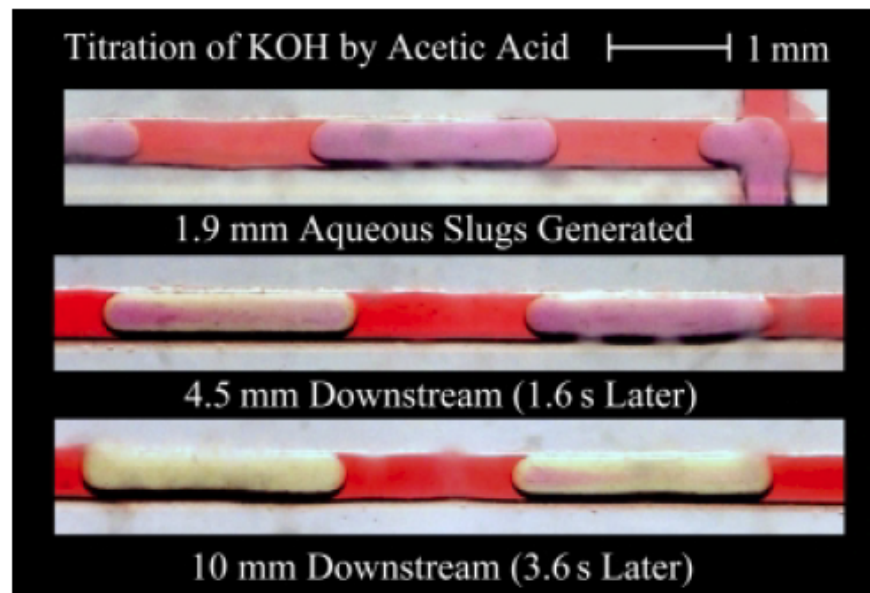


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

(vii) *Claim 27*

2757. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2758. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

2759. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses that

“[a] simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11. From Burns (2001), a POSA would have known that different oils could be used to form the carrier fluid.

2760. A POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* '091 Patent at Table 1 (refractive index of water is 1.3330) to Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

(viii) *Claim 29*

2761. Claim 29 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2762. Claim 29 further recites: “**employing a number of devices in parallel.**”

2763. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2378-2380, demonstrating how Quake discloses employing a number of devices in parallel.

2764. It also would have been obvious to employ a number of devices in parallel based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

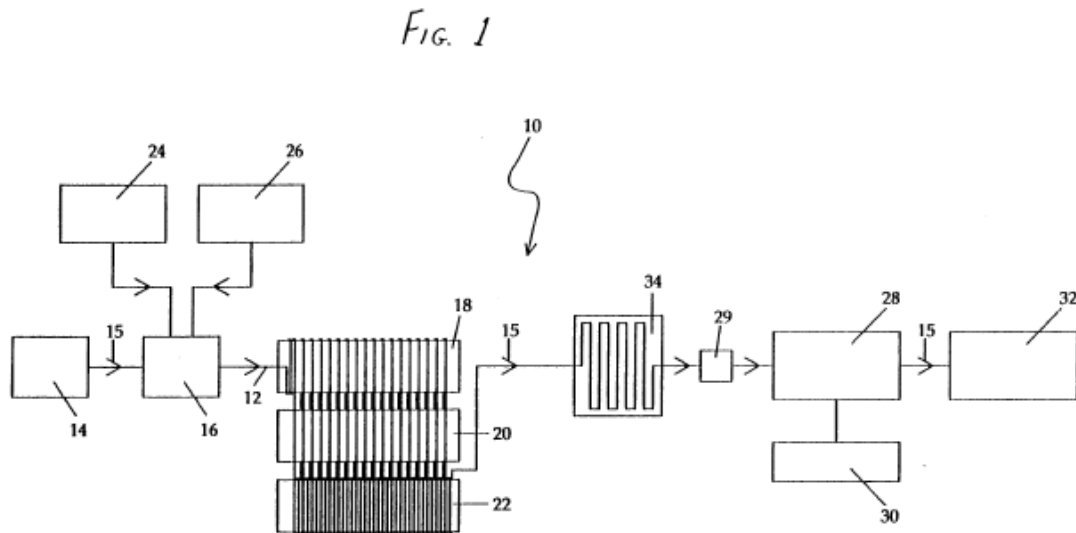
(ix) *Claim 31*

2765. Claim 31 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2766. Claim 31 further recites: “**the reaction is a polymerization reaction.**”

2767. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment

thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2768. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a

syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2769. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2770. It also would have been obvious to conduct a polymerization reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2

(Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

2771. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2772. Claim 33 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.”**

2773. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. *Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel* and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2774. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

2775. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2776. Claim 35 further recites: **“the volume of at least one plug is about 1 femtoliter to about 250 nL.”**

2777. Burns (2001) at least renders obvious this limitation, in light of the background

knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2393-2397, demonstrating how Quake discloses that the volume of at least one plug is about 1 femtoliter to about 250nL.

2778. It also would have been obvious that the volume of at least one plug is about 1 femtoliter to about 250nL based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 36*

2779. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2780. I understand that the Court has not considered whether the preamble of this claim is limiting.

2781. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

2782. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that

conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2783. This reaction is illustrated in Figure 4:

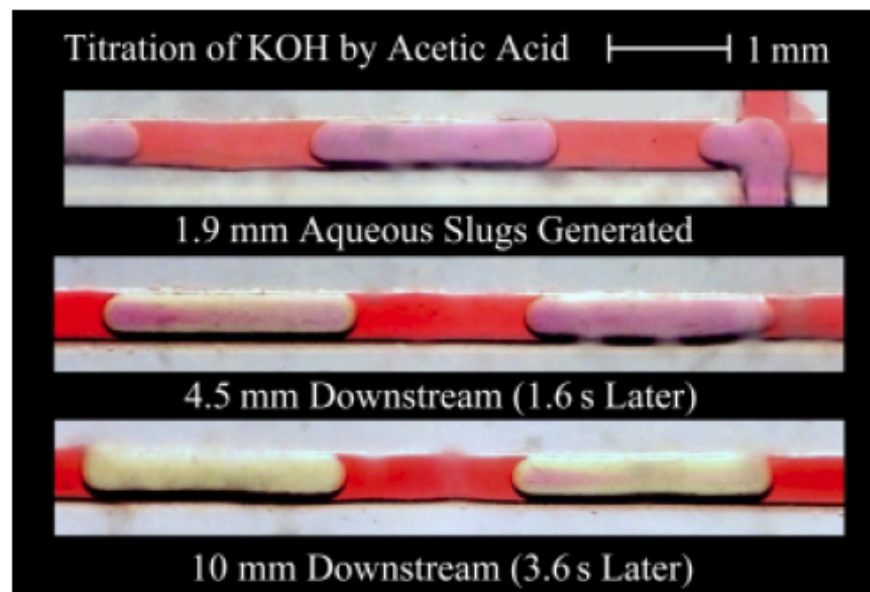
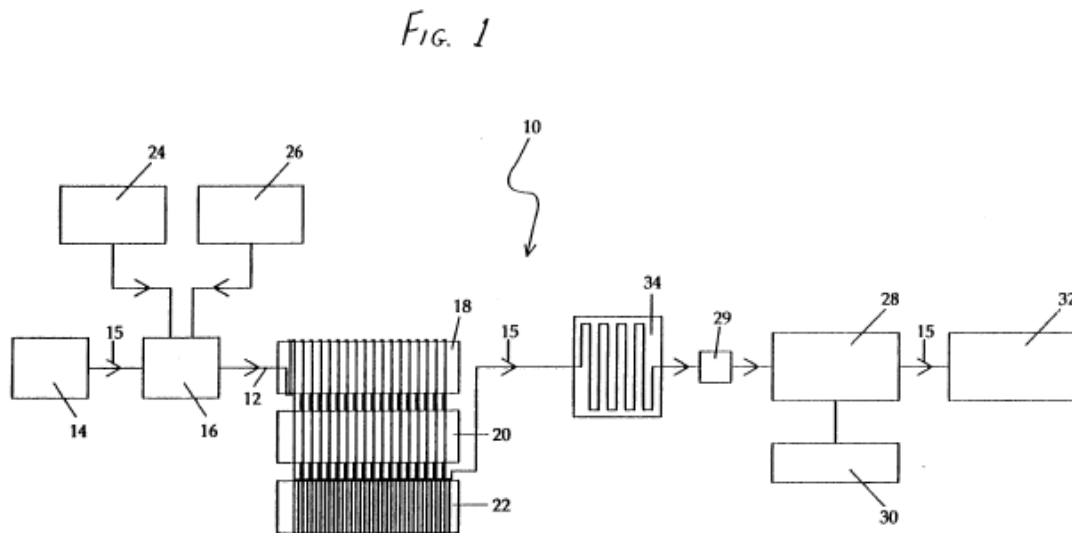


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2784. While it is my opinion that Burns (2001) discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a

sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2785. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template

molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2786. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor

where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2787. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

2788. It also would have been obvious to conduct a reaction within at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2789. Claim 36 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2790. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the

other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2791. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2792. This reaction is illustrated in Figure 4:

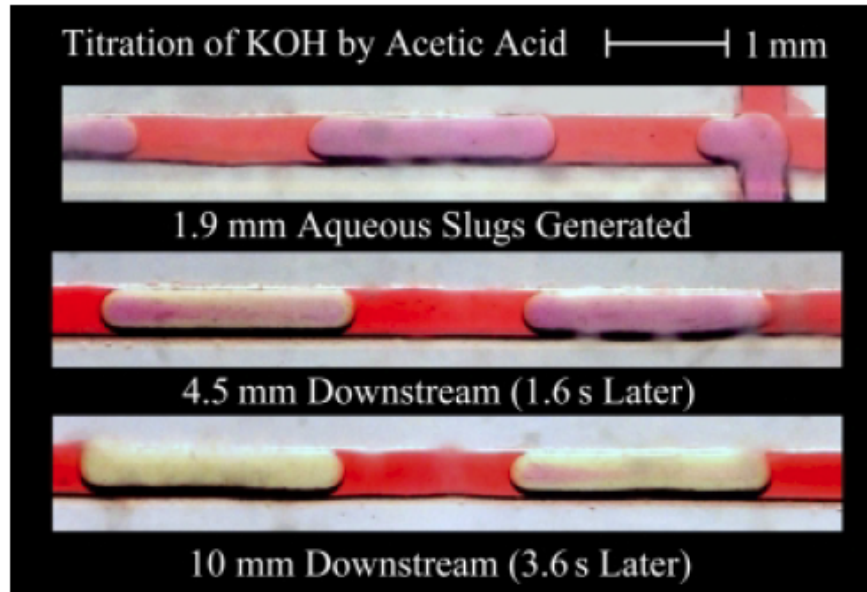


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2793. Claim 36 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2794. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing

into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2795. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2796. This reaction is illustrated in Figure 4:

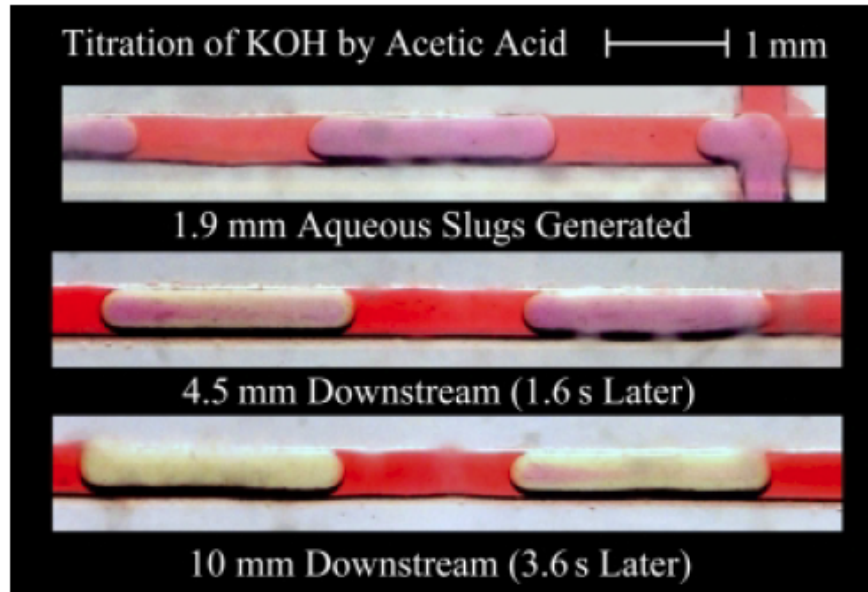


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2797. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with Quake. I incorporate my analysis with respect to ¶¶ 2412-2415, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

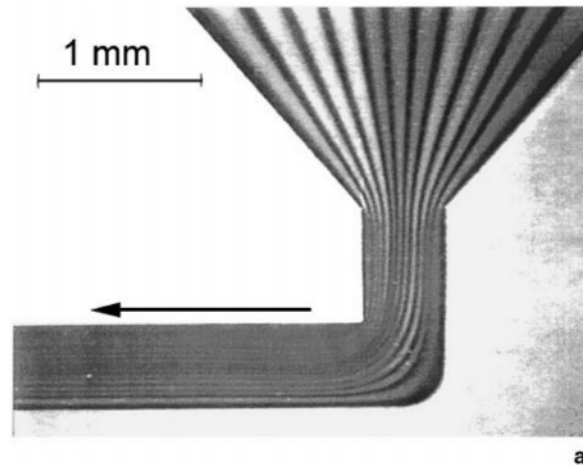
2798. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2799. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in

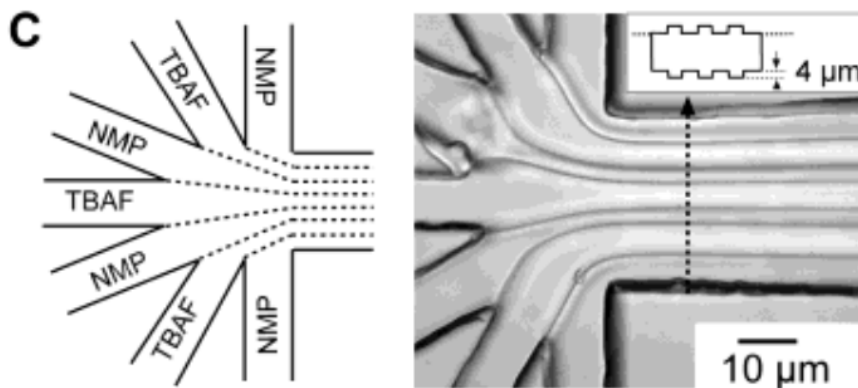
view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2800. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2801. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2802. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

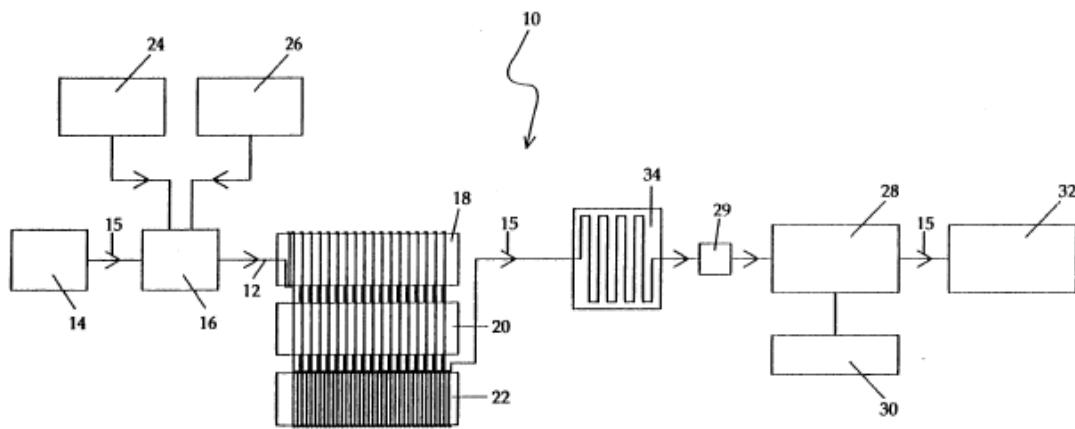


Whitesides at 845-846.

2803. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2804. While it is my opinion that Burns (2001) discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2805. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2806. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2807. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

2808. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2809. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

2810. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

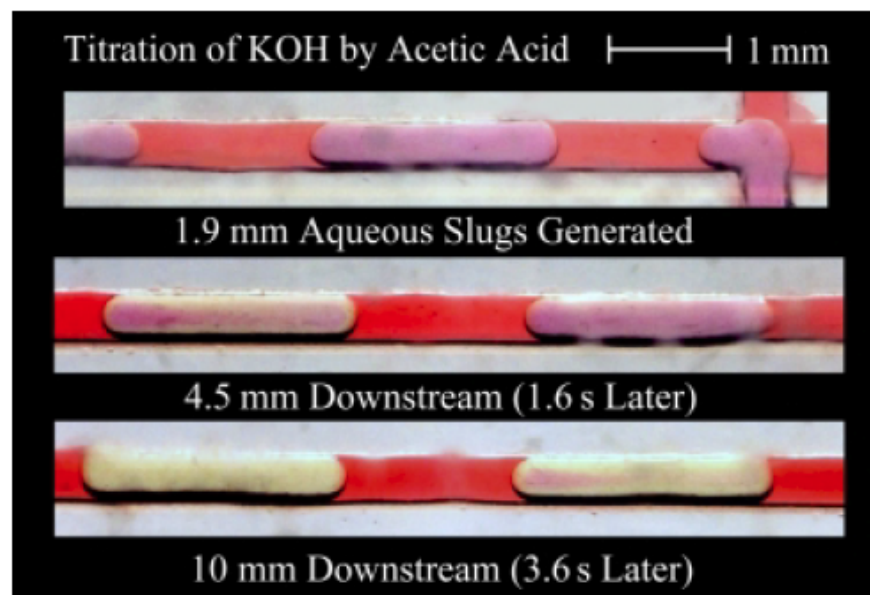


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2811. When a less viscous fluid moves as a “plug” through a more viscous fluid in a

micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

(xiii) *Claim 37*

2812. The preamble of claim 37 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2813. I understand that the Court has not considered whether the preamble of this claim is limiting.

2814. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

2815. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that

conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2816. This reaction is illustrated in Figure 4:

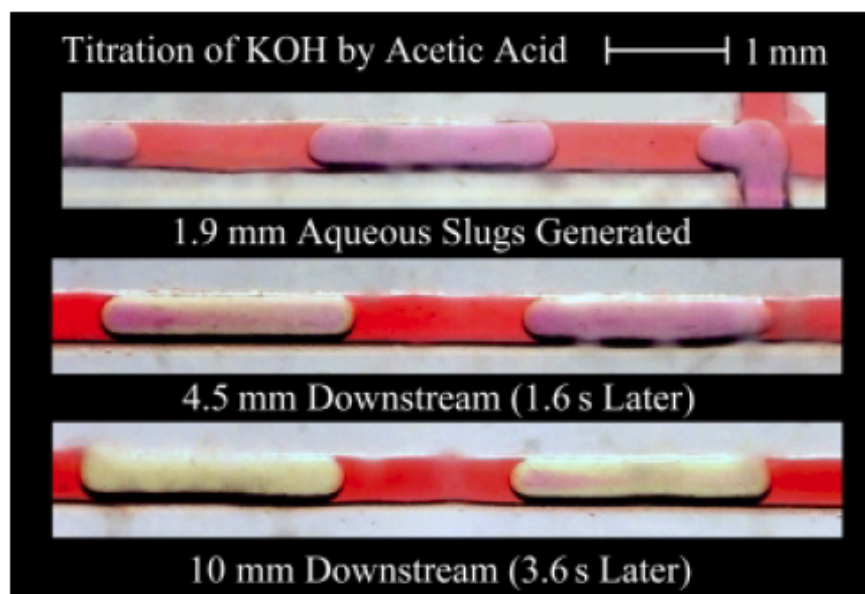


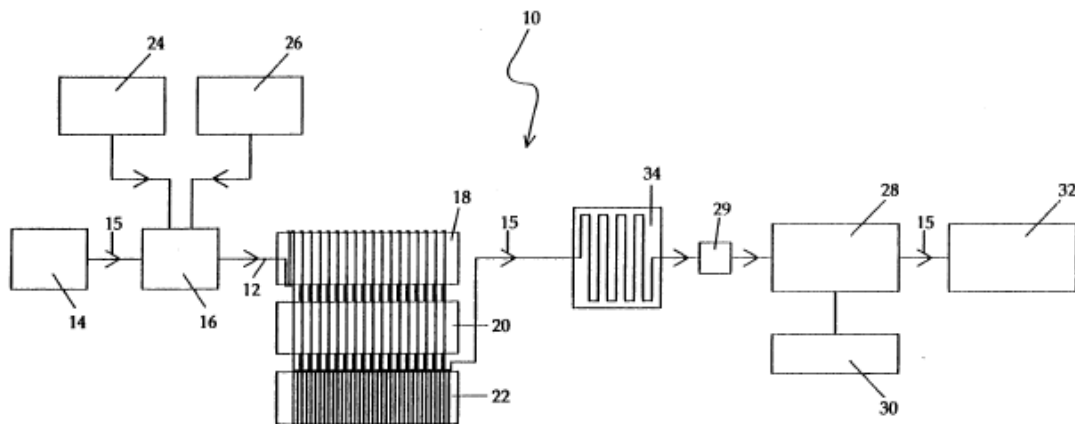
Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2817. While it is my opinion that Burns (2001) discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a

sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2818. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template

molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2819. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor

where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2820. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

2821. It also would have been obvious to conduct a reaction within at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2822. Claim 37 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2823. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the

other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2824. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2825. This reaction is illustrated in Figure 4:

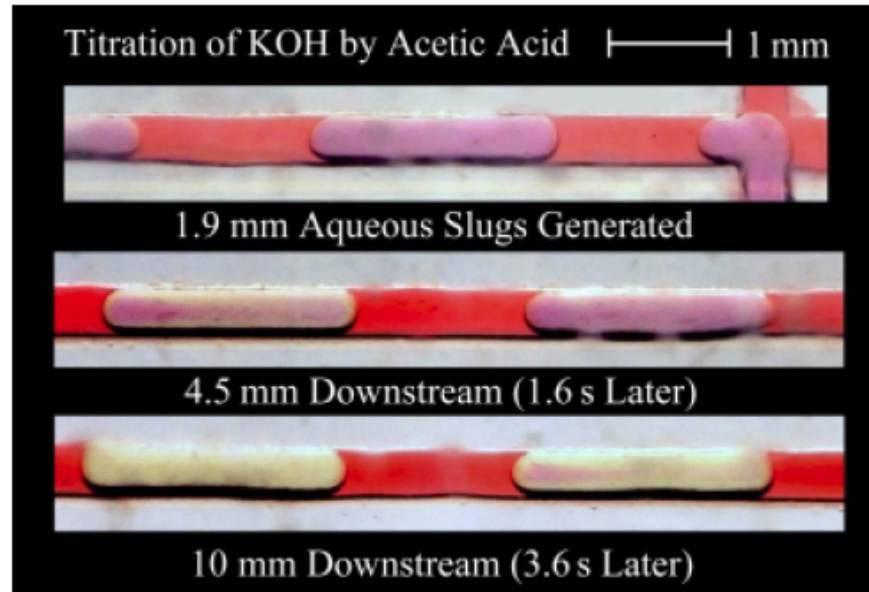


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2826. Claim 37 further recites: “**introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

2827. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases*

through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2828. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2829. This reaction is illustrated in Figure 4:

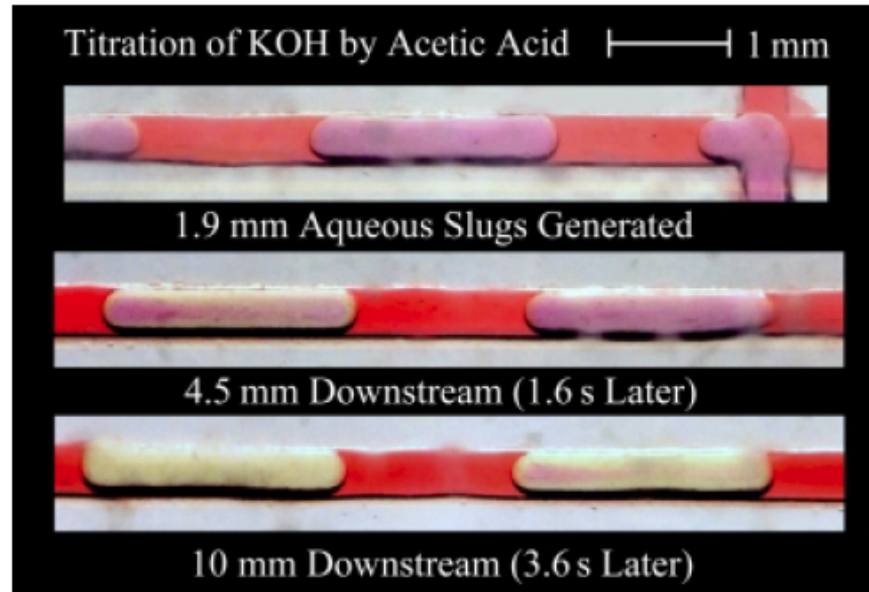


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2830. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with Quake. I incorporate my analysis with respect to ¶¶ 2443-2446, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

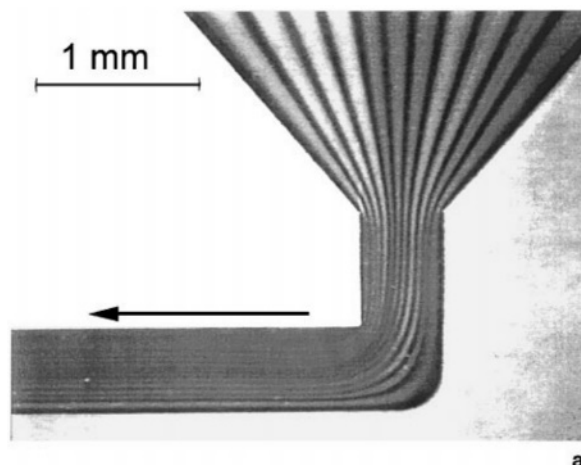
2831. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2832. It also would have been obvious to simultaneously introduce two streams of plug-

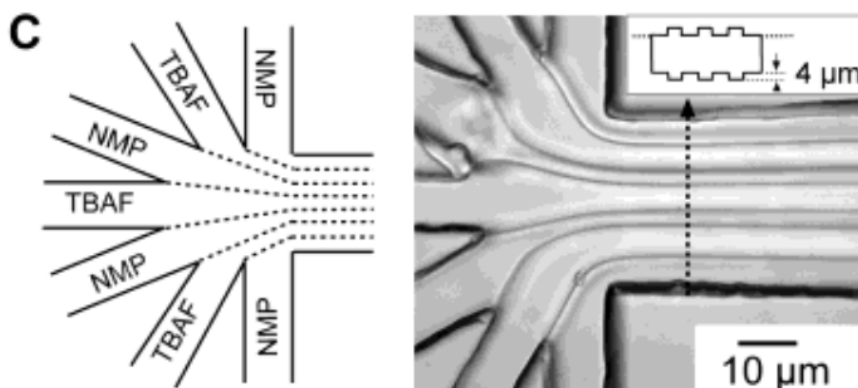
fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2833. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2834. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2835. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

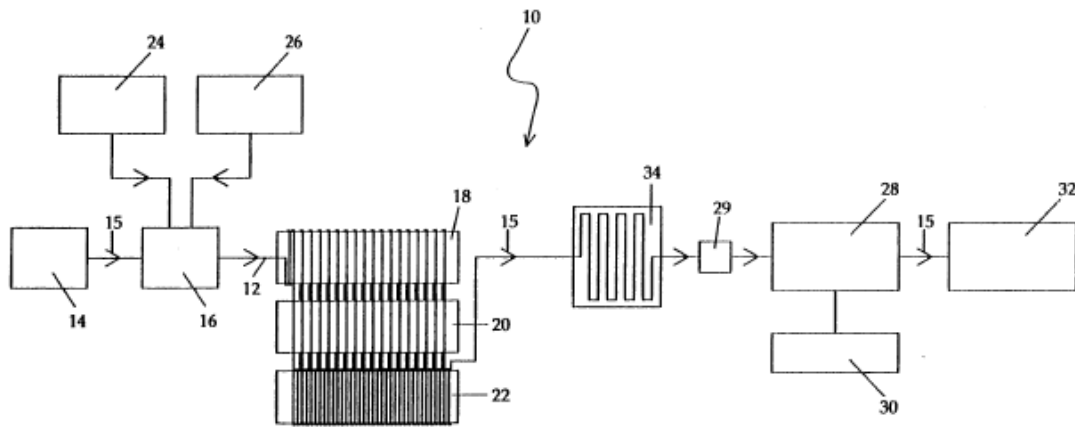


Whitesides at 845-846.

2836. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2837. While it is my opinion that Burns (2001) discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2838. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2839. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2840. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2841. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2842. Claim 37 further recites: **“each plug is substantially surrounded by carrier.”**

2843. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

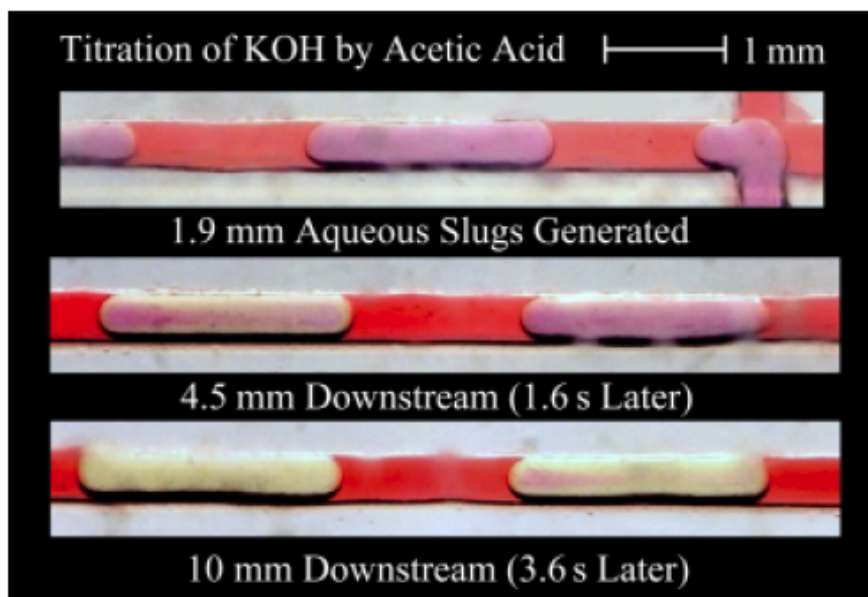


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2844. When a less viscous fluid moves as a “plug” through a more viscous fluid in a

micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

(xiv) *Claim 38*

2845. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2846. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

2847. Burns (2001) satisfies this limitation. For example, Burns (2001) makes clear that the carrier fluid is an oil. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. ***Kerosene was used as the basis of the organic phase*** with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene

to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

(xv) *Claim 39*

2848. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2849. Claim 39 further recites: **“the carrier-fluid comprises at least one surfactant.”**

2850. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2851. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2852. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid.

Id. at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2853. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xvi) *Claim 43*

2854. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2855. Claim 43 further recites: **“the reaction of the plug-fluids forms a soluble reaction product within at least one plug.”**

2856. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses that “[a] simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2857. This reaction is illustrated in Figure 4:

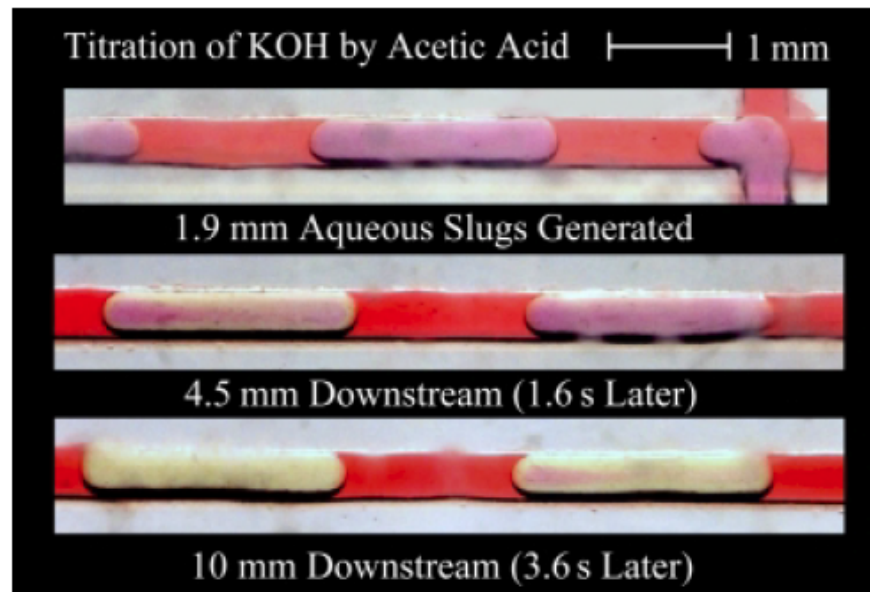


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

(xvii) *Claim 53*

2858. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2859. Claim 53 further recites: “**employing a number of devices in parallel.**”

2860. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2474-2477, demonstrating how Quake discloses employing a number of devices in parallel.

2861. It also would have been obvious to employ a number of devices in parallel based

on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) *Claim 56*

2862. Claim 56 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

2863. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

2864. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2478-2482, demonstrating how Quake discloses that the volume of at least one plug is about 1 femoliter to about 250nL.

2865. It also would have been obvious that the volume of at least one plug is about 1 femtoliter to about 250nL based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xix) *Claim 57*

2866. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2867. I understand that the Court has not considered whether the preamble of this claim

is limiting.

2868. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

2869. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

2870. This reaction is illustrated in Figure 4:

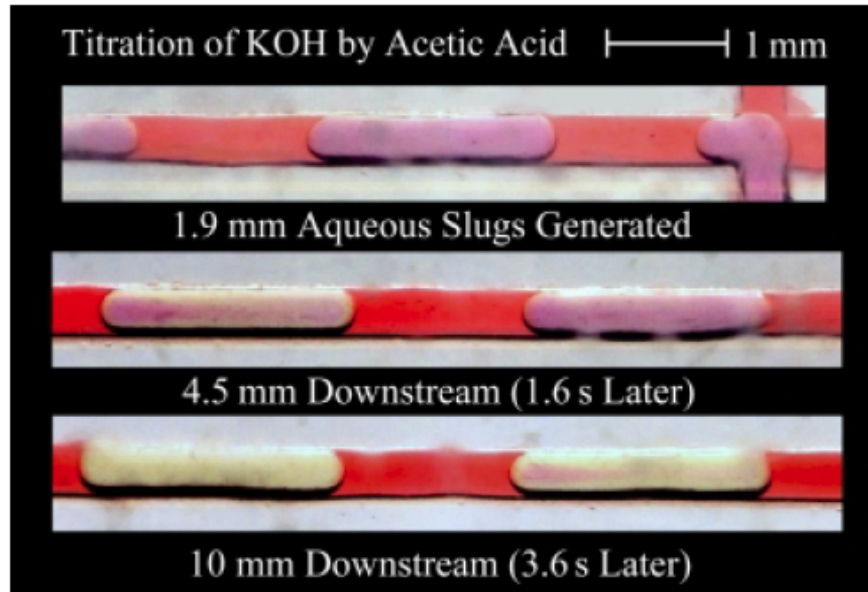
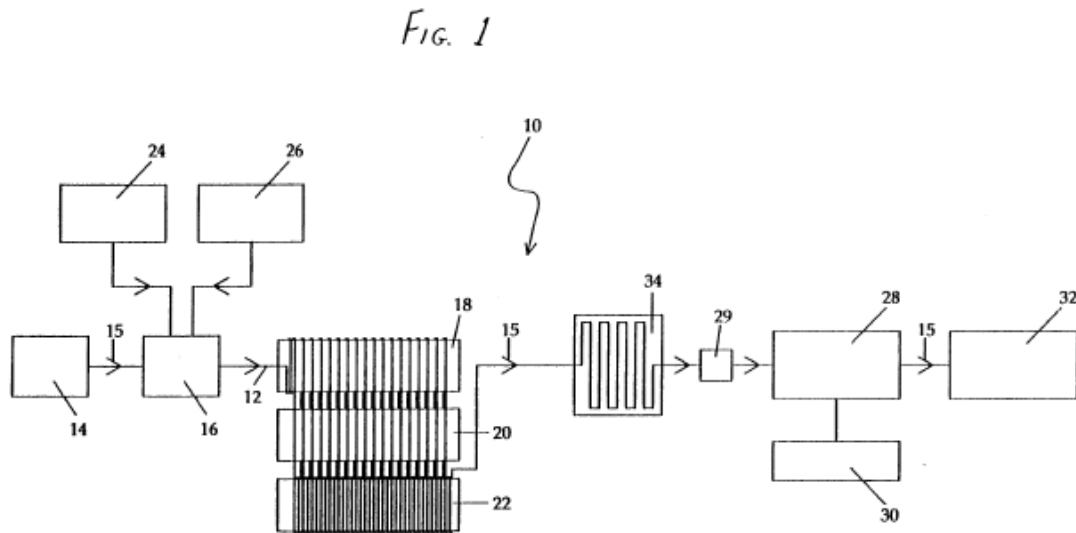


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2871. While it is my opinion that Burns (2001) discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into

tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2872. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from

the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2873. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2874. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may

react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2875. It also would have been obvious to conduct a reaction within at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2876. Claim 57 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2877. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2878. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a

saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2879. This reaction is illustrated in Figure 4:

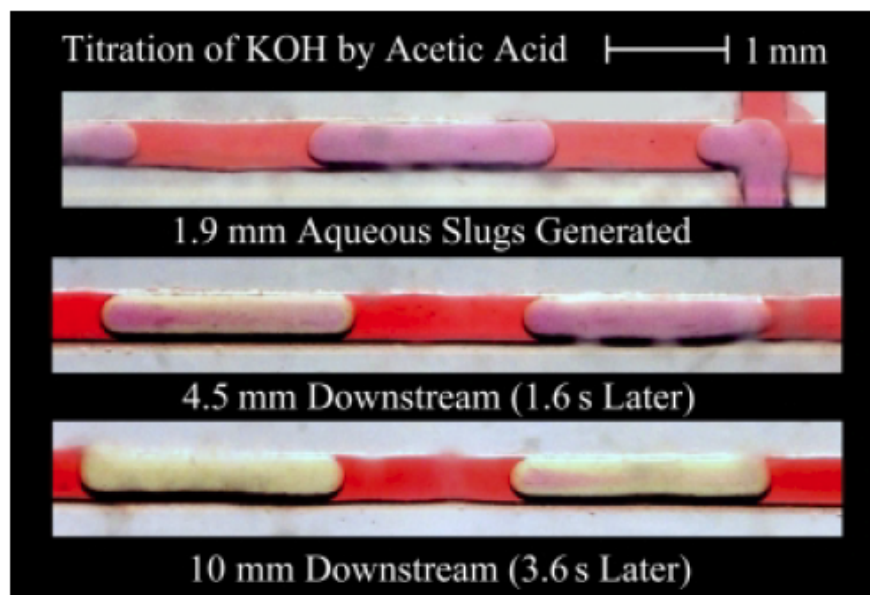


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2880. Claim 57 further recites: “**introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible**

with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

2881. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2882. Burns (2001) also makes clear that aqueous fluid is used to conduct the reactions within slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

2883. This reaction is illustrated in Figure 4:

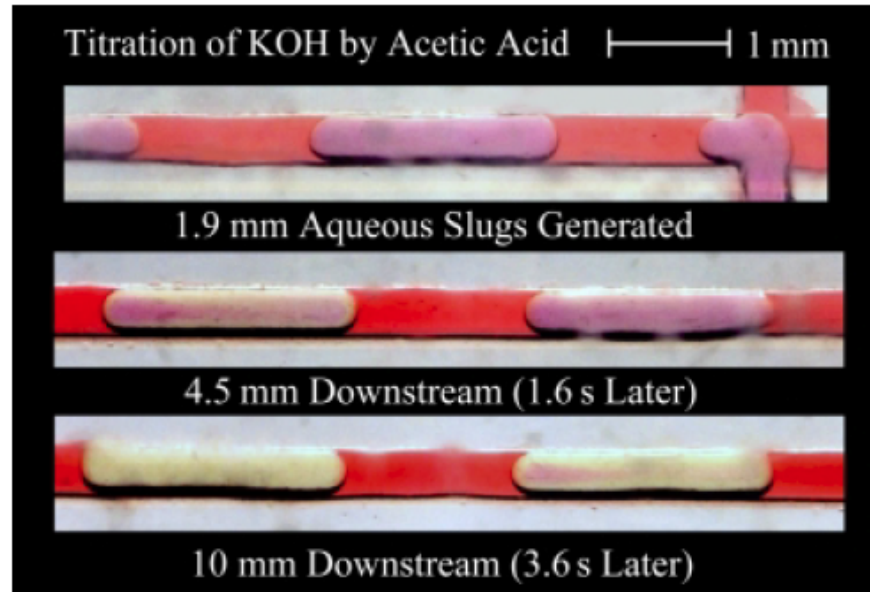


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2884. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with Quake. I incorporate my analysis with respect to ¶¶ 2497-2500, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

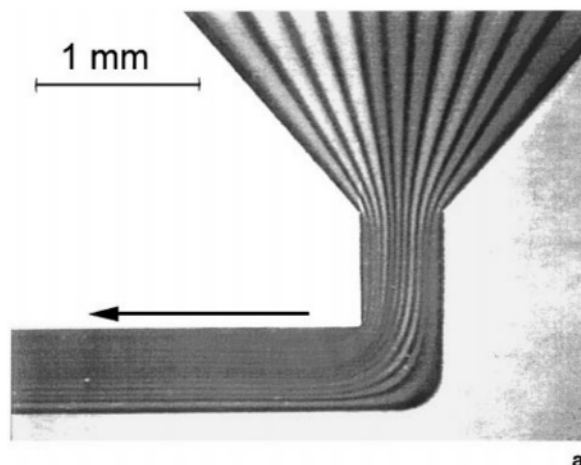
2885. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

2886. It also would be obvious to simultaneously introduce two streams of plug-

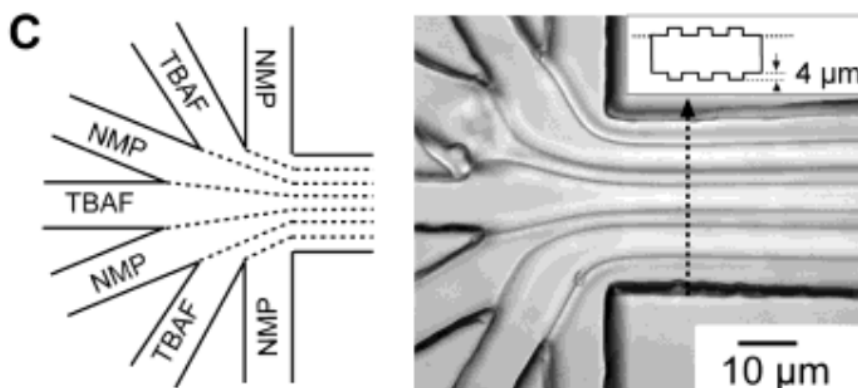
fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2887. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2888. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2889. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

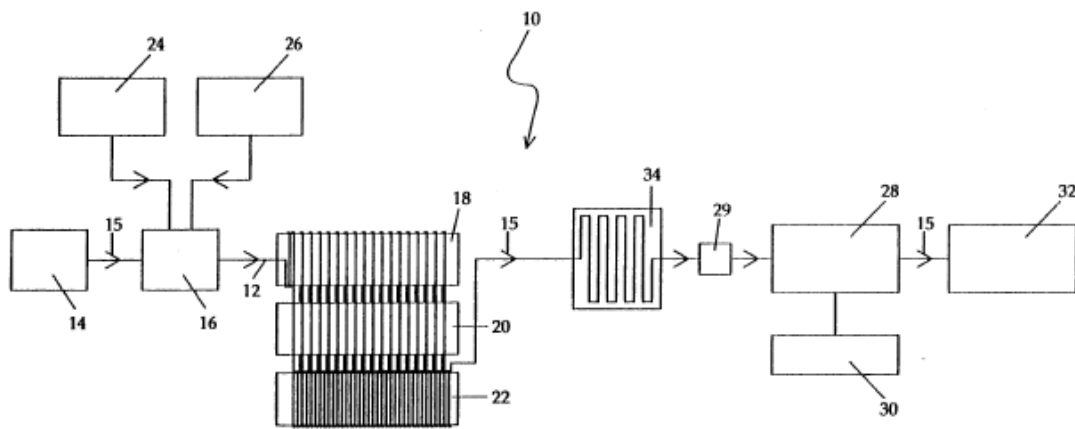


Whitesides at 845-846.

2890. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2891. While it is my opinion that Burns (2001) discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2892. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2893. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2894. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2895. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2896. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

2897. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

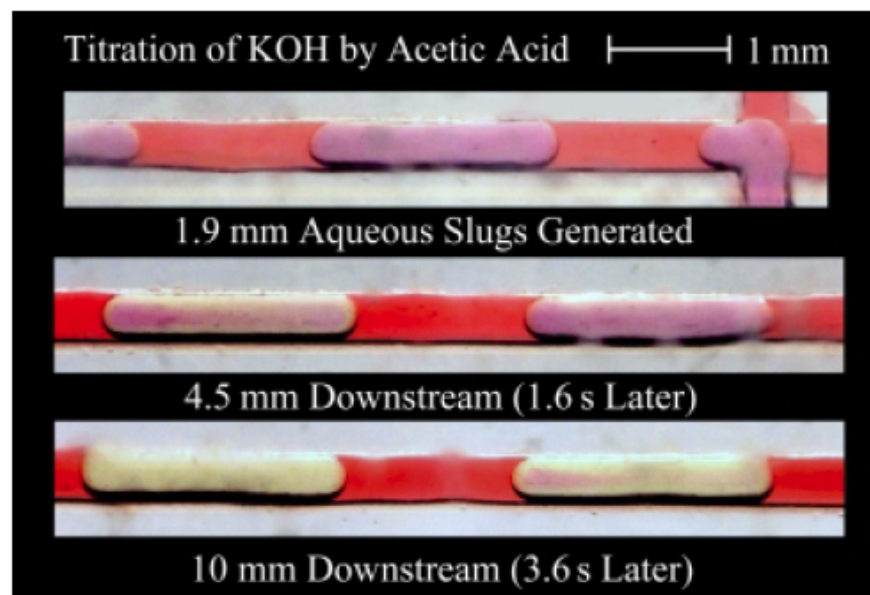


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

2898. When a less viscous fluid moves as a “plug” through a more viscous fluid in a

micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

(xx) *Claim 58*

2899. Claim 58 of the '091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

2900. Claim 58 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area.”**

2901. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. *Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel* and reversing the process.” Burns (2001) at 10-11 (emphasis added).

2902. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles

and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(d) Invalidity Based on Nisisako

2903. It is my opinion that Nisisako discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

2904. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2905. I understand that the Court has not considered whether the preamble of this claim is limiting.

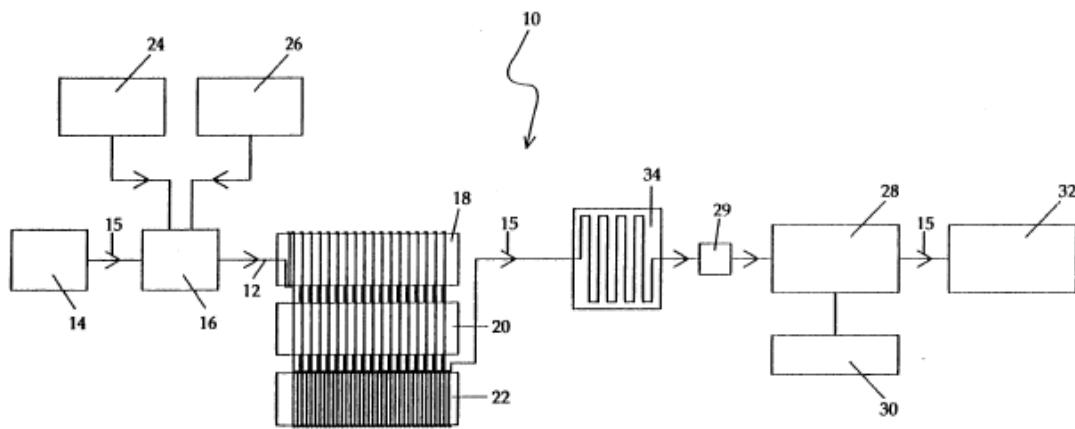
2906. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network*.” Nisisako at Abstract (emphasis added).

2907. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a

manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

2908. While it is my opinion that Nisisako discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2909. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2910. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2911. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2912. It also would have been obvious to conduct a reaction within at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2913. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2914. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

2915. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both**

the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

2916. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

2917. The figures in Nisisako also disclose this limitation:

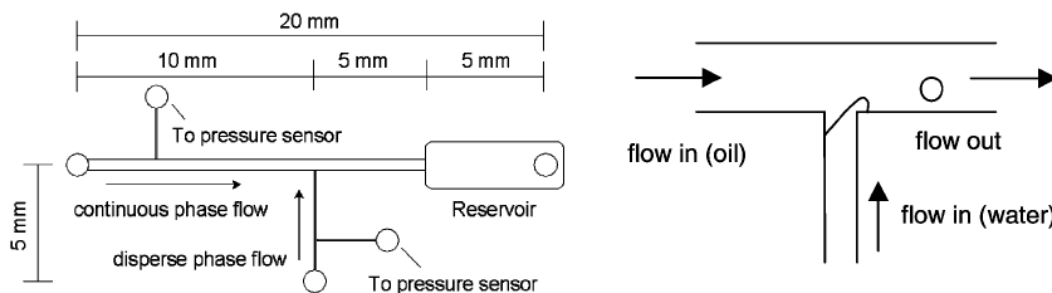


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

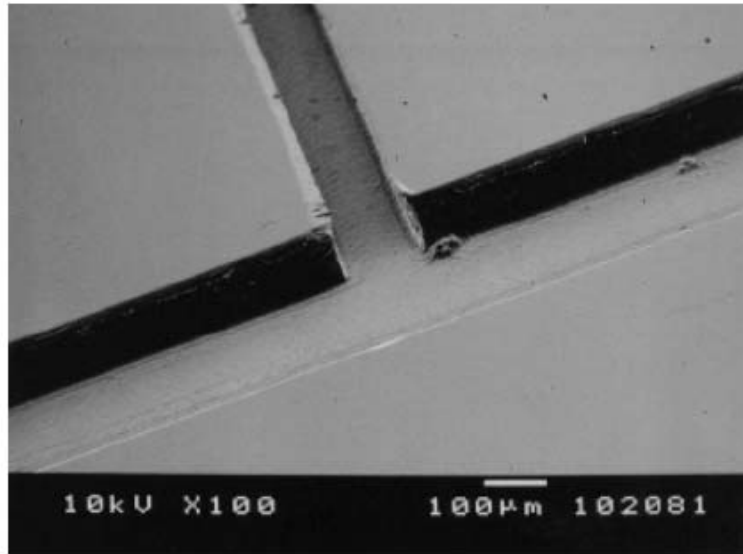


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

2918. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

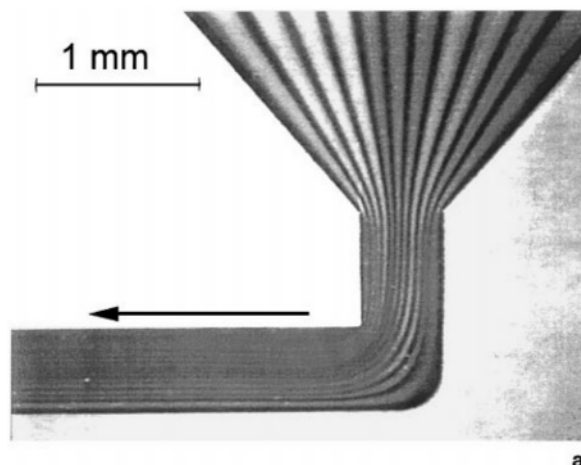
2919. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Nisisako with Quake. I incorporate my analysis with respect to ¶¶ 2332-2335, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

2920. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid streams are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

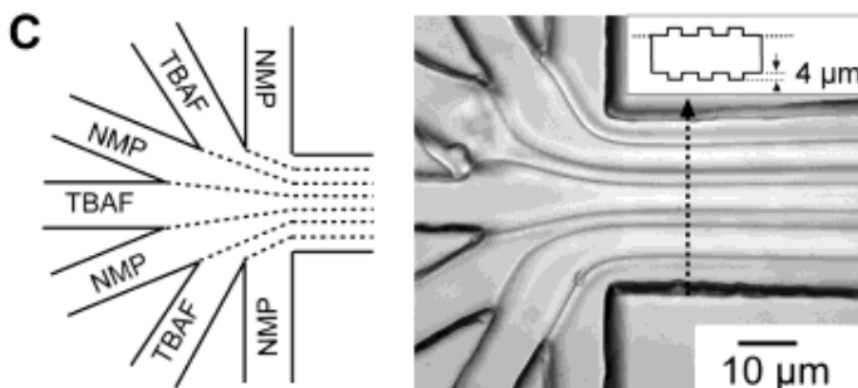
2921. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2922. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2923. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2924. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

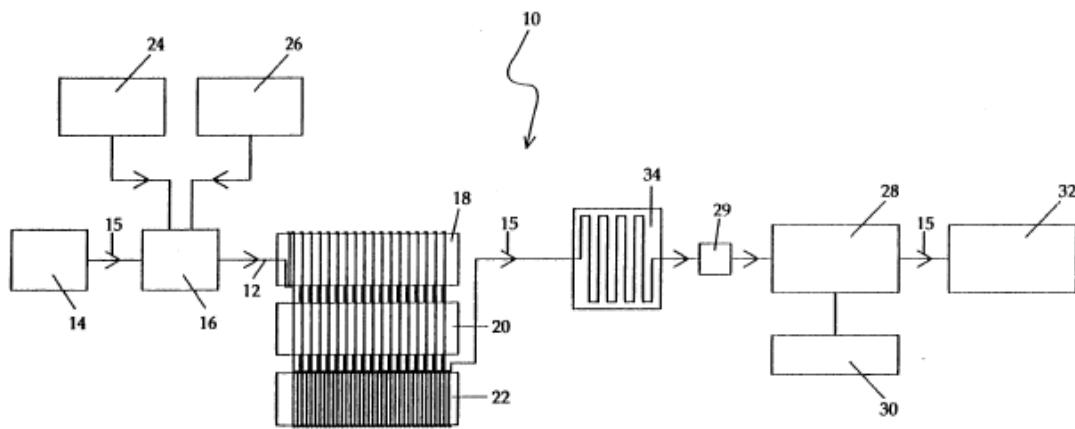


Whitesides at 845-846.

2925. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2926. While it is my opinion that Nisisako discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2927. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2928. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2929. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2930. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2931. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

2932. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by carrier fluid:

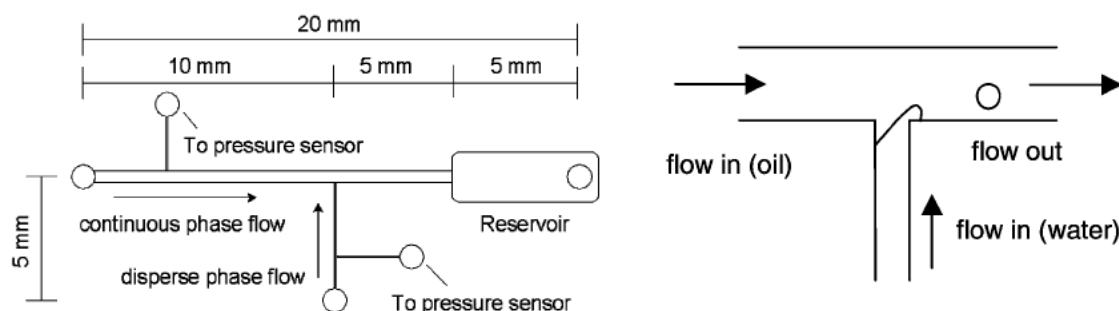


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

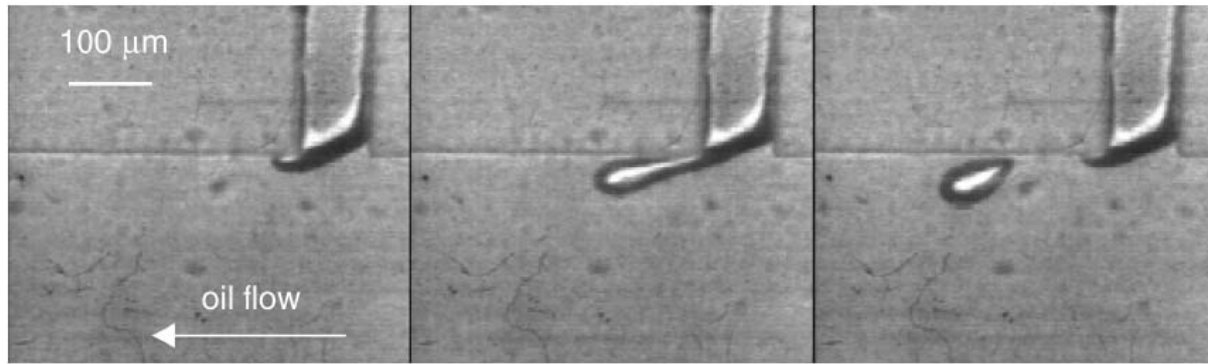


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

(ii) *Claim 2*

2933. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2934. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

2935. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); see also Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating *water-in-oil* droplets in a microchannel network.”).

(iii) *Claim 3*

2936. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2937. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

2938. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2939. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2940. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction

fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2941. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

2942. Claim 5 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2943. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

2944. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

2945. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or

surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

2946. It also would have been obvious to use a carrier-fluid comprising a surfactant view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

2947. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 6*

2948. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2949. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

2950. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and *water as the dispersed phase*, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis

added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. ***Ultra-pure water is used as the dispersed phase*** and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating ***water-in-oil*** droplets in a microchannel network.”).

(vi) *Claim 11*

2951. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2952. Claim 11 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

2953. Nisisako satisfies this limitation. For example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24.

(vii) *Claim 27*

2954. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2955. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

2956. Nisisako satisfies this limitation. For example, Nisisako describes that “[s]yringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.*” Nisisako at 25. From Nisisako, a POSA would have known that different oils could be used to form the carrier fluid.

2957. A POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* ’091 Patent at Table 1 (refractive index of water is 1.3330) *to* Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

(viii) *Claim 29*

2958. Claim 29 of the ’091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2959. Claim 29 further recites: “**employing a number of devices in parallel.**”

2960. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2378-2380, demonstrating how Quake discloses employing a number of devices in parallel.

2961. It also would have been obvious to employ a number of devices in parallel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited

therein.

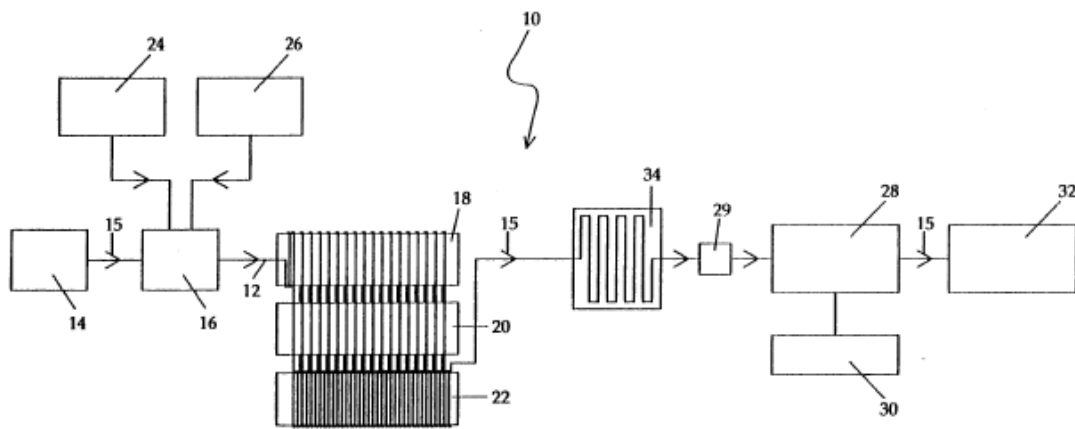
(ix) *Claim 31*

2962. Claim 31 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2963. Claim 31 further recites: **“the reaction is a polymerization reaction.”**

2964. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” See Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 µl, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2965. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2966. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2967. It also would have been obvious to conduct a polymerization reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

2968. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2969. Claim 33 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.”**

2970. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep. For given experimental parameters, regular-sized droplets are reproducibly formed at a uniform speed. The diameter of these droplets is controllable in the range from 100–380 μm as the flow velocity of the continuous phase is varied from 0.01 m s^{-1} to 0.15 m s^{-1} .” Nisisako at Abstract.

2971. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

2972. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

2973. Claim 35 further recites: **“the volume of at least one plug is about 1 femtoliter to about 250 nL.”**

2974. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract.

2975. Nisisako also describes that “[t]he droplet size can be controlled: the minimum

diameter of the droplets was about 100 μm , and the maximum 380 μm , as the flow velocity of the continuous phase was changed from 0.01 to 0.15 m s^{-1} .” Nisisako at 26. In Figure 6, Nisisako plots volume against velocity, and shows that all velocities tested, the volume of a droplet was below 250 nL:

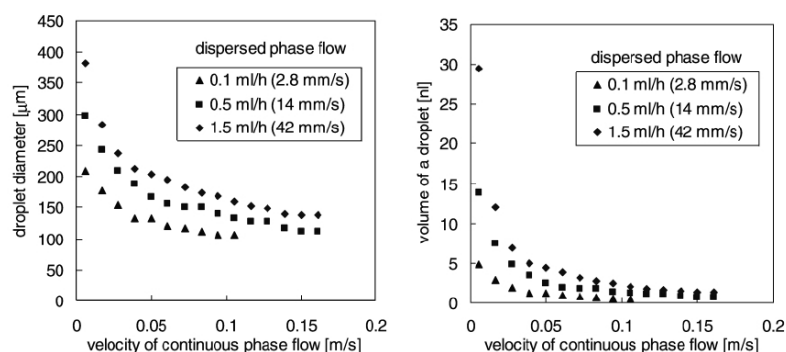


Fig. 6 Effect of velocity of continuous phase flow on droplet size (left: droplet diameter data, right: volume of a droplet calculated from the diameter).

Nisisako at Fig. 6.

(xii) *Claim 36*

2976. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

2977. I understand that the Court has not considered whether the preamble of this claim is limiting.

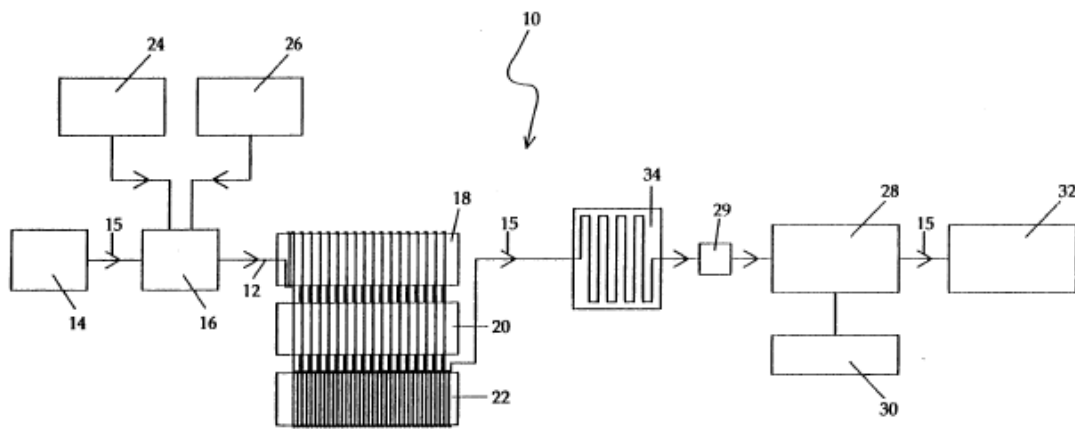
2978. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network*.” Nisisako at Abstract (emphasis added).

2979. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be

decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

2980. While it is my opinion that Nisisako discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2981. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2982. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2983. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

2984. It also would have been obvious to conduct a reaction within at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2985. Claim 36 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

2986. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

2987. Claim 36 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible**

with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

2988. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

2989. The figures in Nisisako also disclose this limitation:

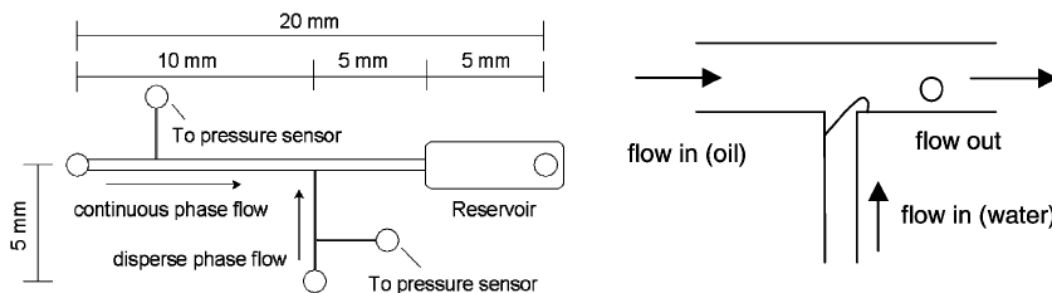


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

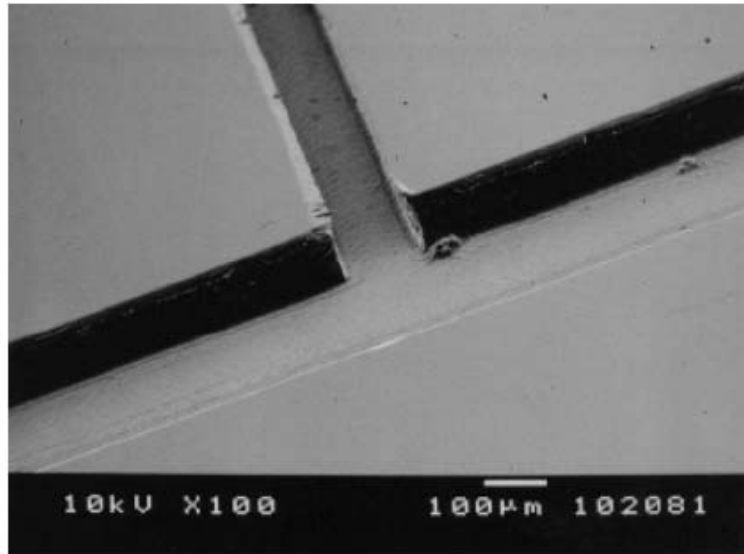


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

2990. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

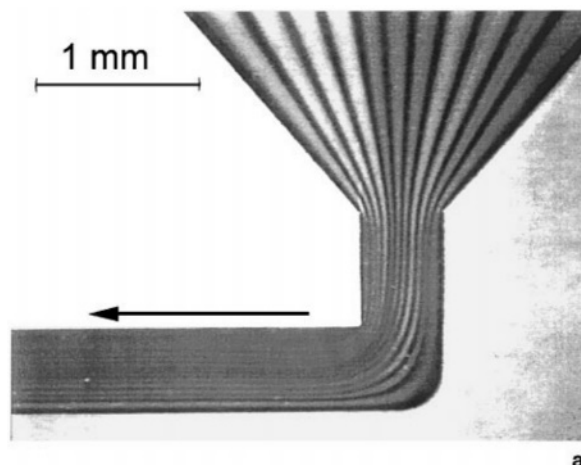
2991. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Nisisako with Quake. I incorporate my analysis with respect to ¶¶ 2412-2415, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

2992. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid streams are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

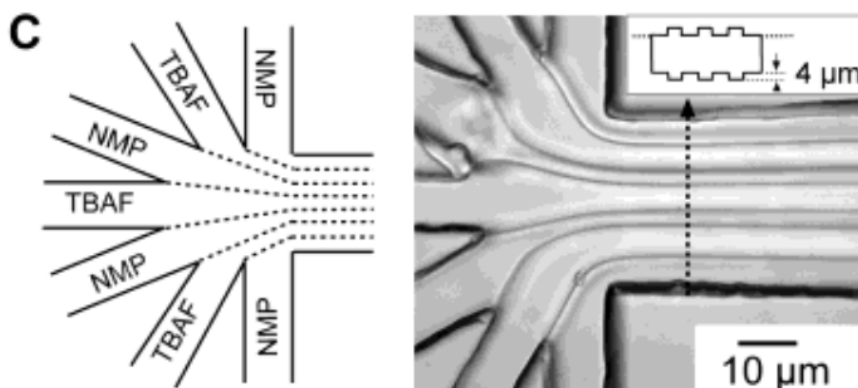
2993. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, ***chemical reactions***, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

2994. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

2995. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



2996. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

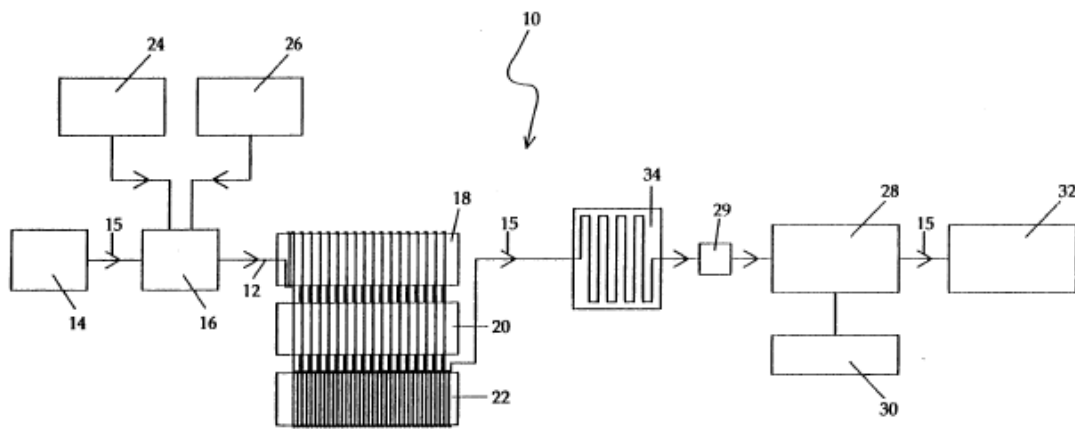


Whitesides at 845-846.

2997. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2998. While it is my opinion that Nisisako discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

2999. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3000. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3001. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3002. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3003. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

3004. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by carrier fluid:

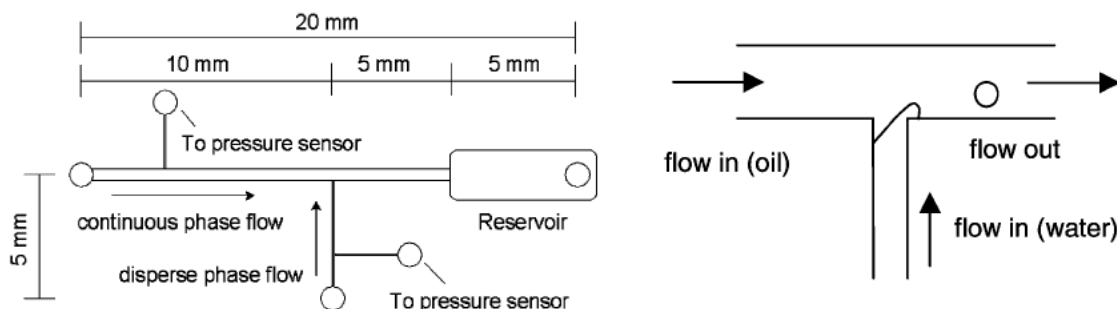


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

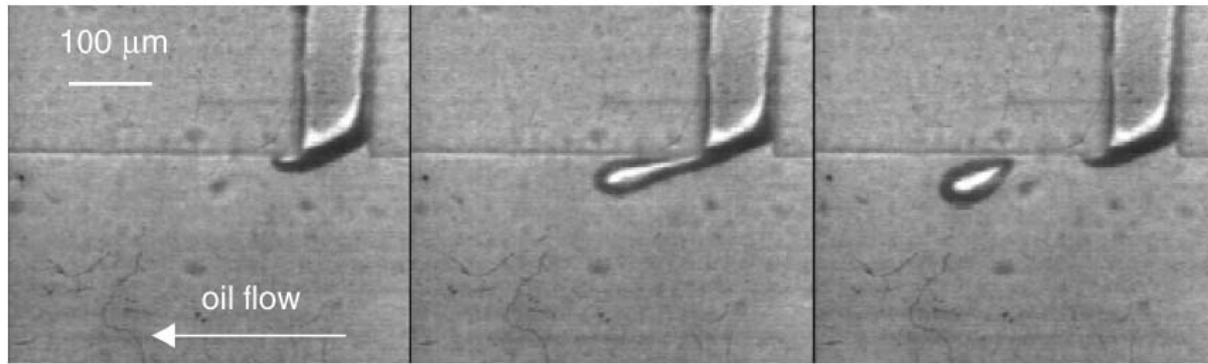


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

(xiii) *Claim 37*

3005. The preamble of claim 37 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3006. I understand that the Court has not considered whether the preamble of this claim is limiting.

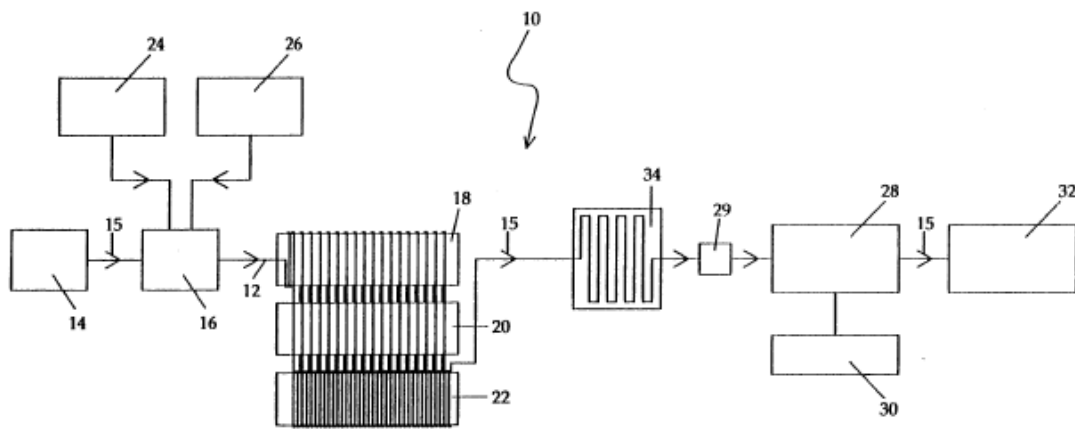
3007. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network.*” Nisisako at Abstract (emphasis added).

3008. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a

manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

3009. While it is my opinion that Nisisako discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3010. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3011. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3012. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3013. It also would have been obvious to conduct a reaction within at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3014. Claim 37 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3015. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

3016. Claim 37 further recites: “**introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a**

second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

3017. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

3018. The figures in Nisisako also disclose this limitation:

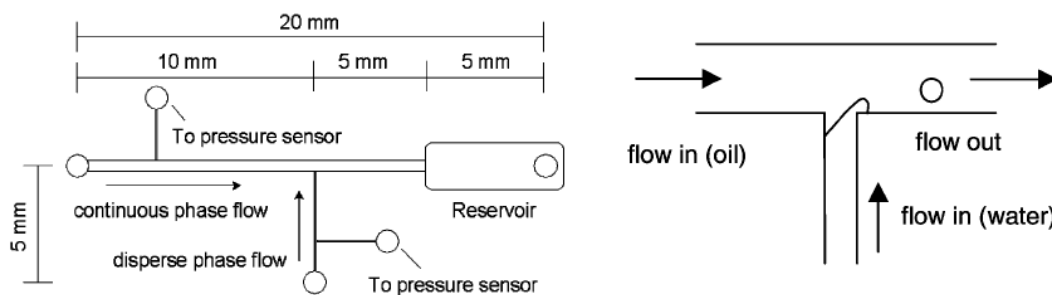


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

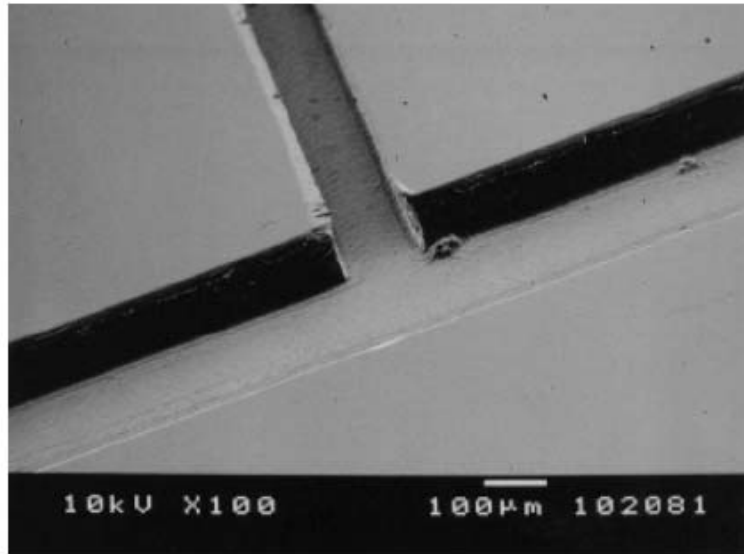


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

3019. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

3020. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Nisisako with Quake. I incorporate my analysis with respect to ¶¶ 2443-2446, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

3021. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams.

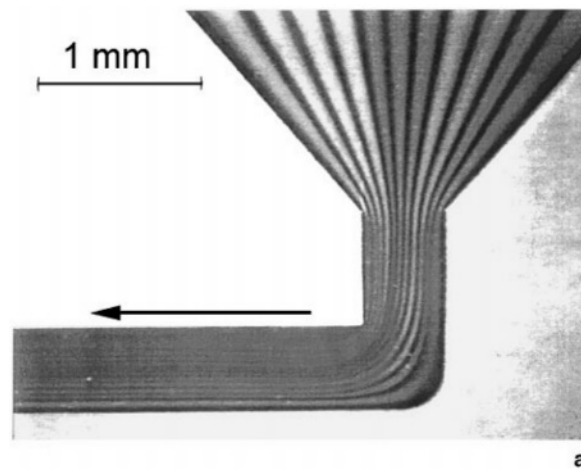
Kenis at 83.

3022. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

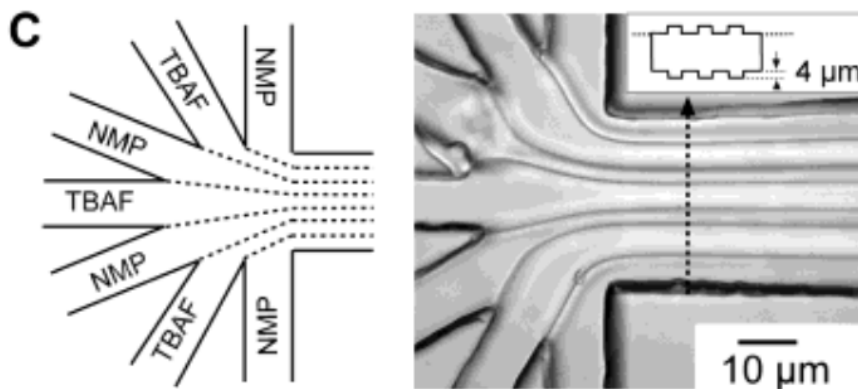
3023. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3024. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix.

Figure 4a of Erbacher is reproduced below:



3025. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

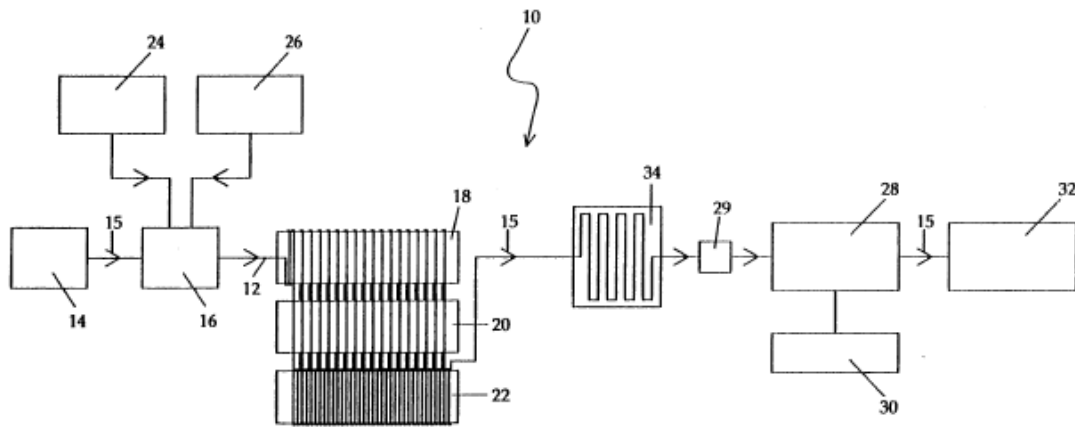


Whitesides at 845-846.

3026. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3027. While it is my opinion that Nisisako discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3028. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3029. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3030. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3031. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3032. Claim 37 further recites: “**each plug is substantially surrounded by carrier.**”

3033. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by carrier fluid:

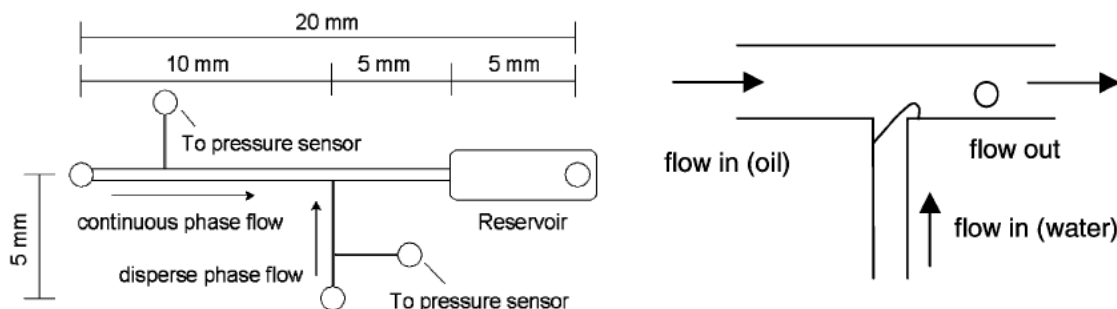


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

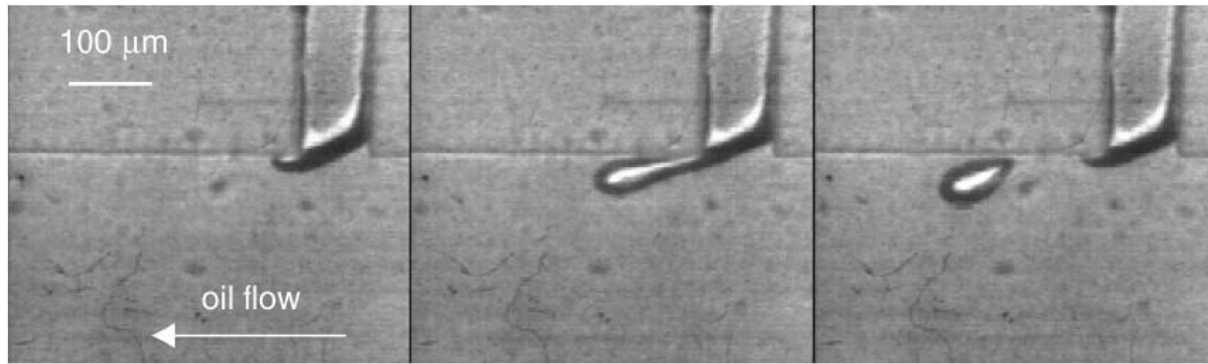


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

(xiv) *Claim 38*

3034. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3035. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

3036. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and **high oleic sunflower oil (triolein, 80%) as the continuous phase**. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”); Nisisako at 24 (“We propose here a novel method for generating **water-in-oil** droplets in a microchannel network.”).

(xv) *Claim 39*

3037. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3038. Claim 39 further recites: **“the carrier-fluid comprises at least one surfactant.”**

3039. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3040. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3041. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they

have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3042. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xvi) *Claim 43*

3043. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3044. Claim 43 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

3045. Nisisako satisfies this limitation. For example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24.

(xvii) *Claim 53*

3046. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3047. Claim 53 further recites: “**employing a number of devices in parallel.**”

3048. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2474-2477, demonstrating how Quake discloses employing a number of devices in parallel.

3049. It also would have been obvious to employ a number of devices in parallel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) *Claim 56*

3050. Claim 56 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3051. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

3052. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract.

3053. Nisisako also describes that “[t]he droplet size can be controlled: the minimum diameter of the droplets was about 100 μm , and the maximum 380 μm , as the flow velocity of the continuous phase was changed from 0.01 to 0.15 m s^{-1} .” Nisisako at 26. In Figure 6, Nisisako plots volume against velocity, and shows that all velocities tested, the volume of a droplet was

below 250 nL:

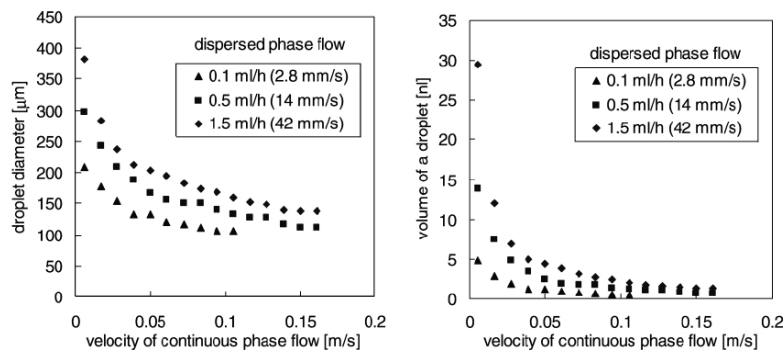


Fig. 6 Effect of velocity of continuous phase flow on droplet size (left: droplet diameter data, right: volume of a droplet calculated from the diameter).

Nisisako at Fig. 6.

(xix) *Claim 57*

3054. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3055. I understand that the Court has not considered whether the preamble of this claim is limiting.

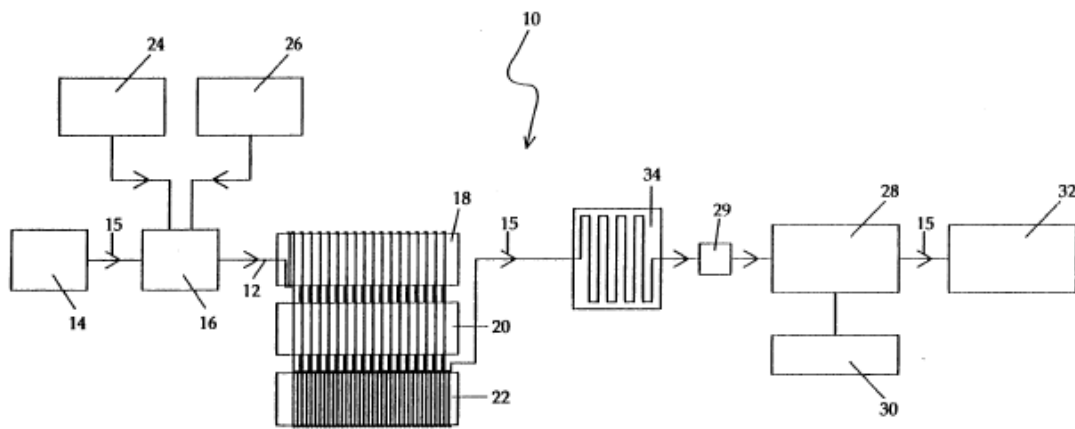
3056. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network.*” Nisisako at Abstract (emphasis added).

3057. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a

manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

3058. While it is my opinion that Nisisako discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3059. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3060. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3061. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5.

3062. It also would have been obvious to conduct a reaction within at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3063. Claim 57 further recites: **“introducing a carrier-fluid into a first microchannel of a device.”**

3064. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semiconductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

3065. Claim 57 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the**

reaction of the reagents substantially occurs in the plug.”

3066. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

3067. The figures in Nisisako also disclose this limitation:

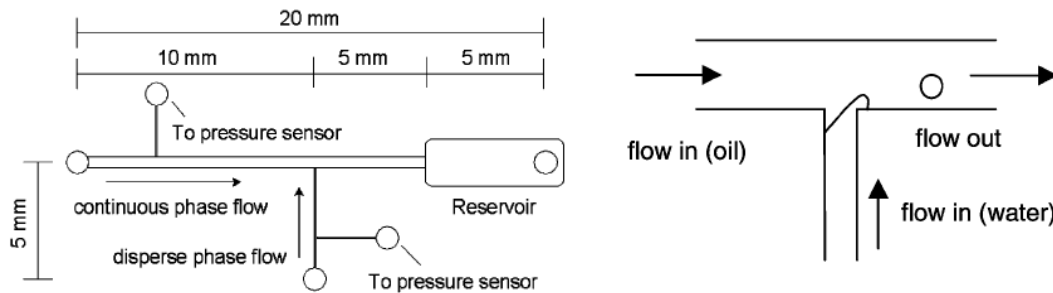


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

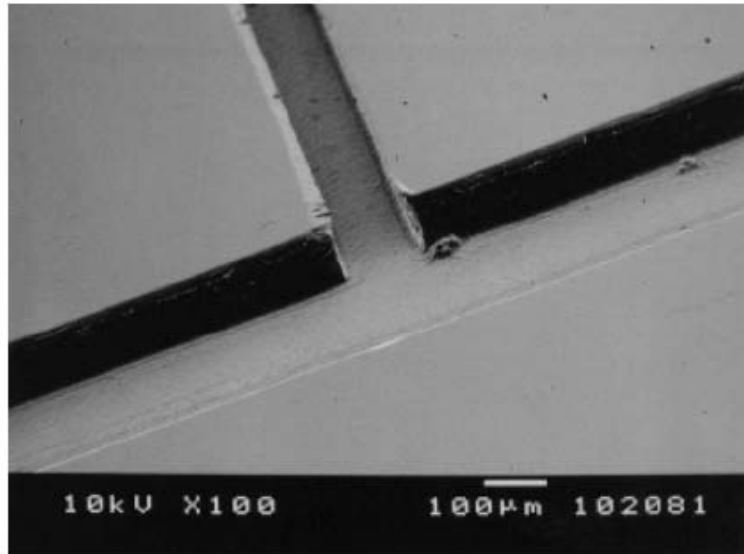


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

3068. Nisisako also makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

3069. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Nisisako with Quake. I incorporate my analysis with respect to ¶¶ 2497-2500, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

3070. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams.

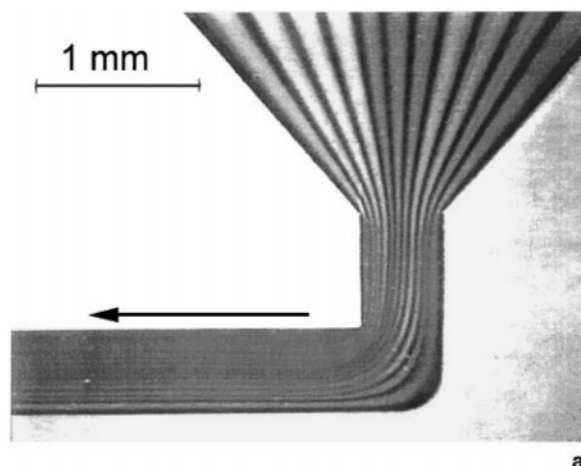
Kenis at 83.

3071. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

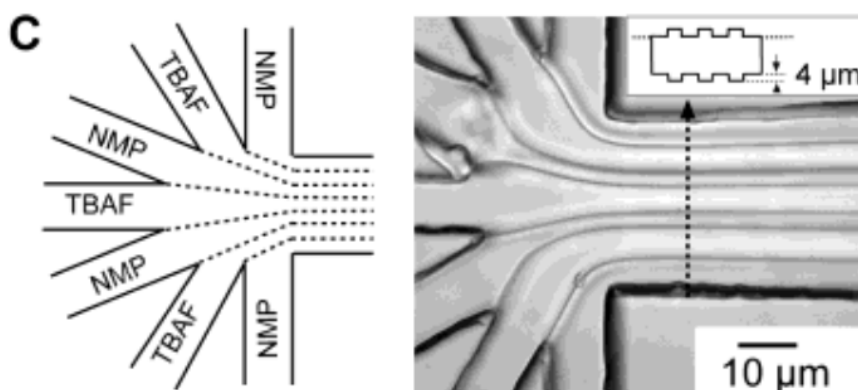
3072. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3073. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix.

Figure 4a of Erbacher is reproduced below:



3074. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

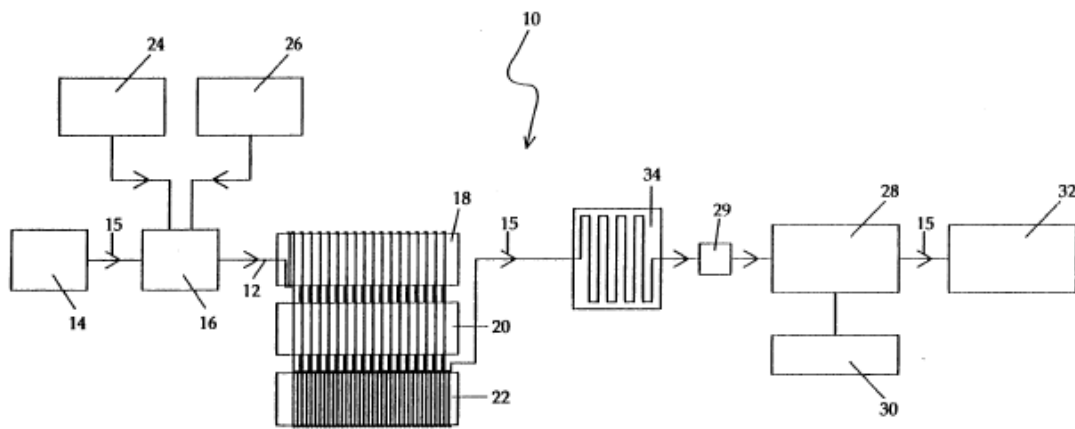


Whitesides at 845-846.

3075. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3076. While it is my opinion that Nisisako discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3077. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3078. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3079. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3080. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3081. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

3082. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by carrier fluid:

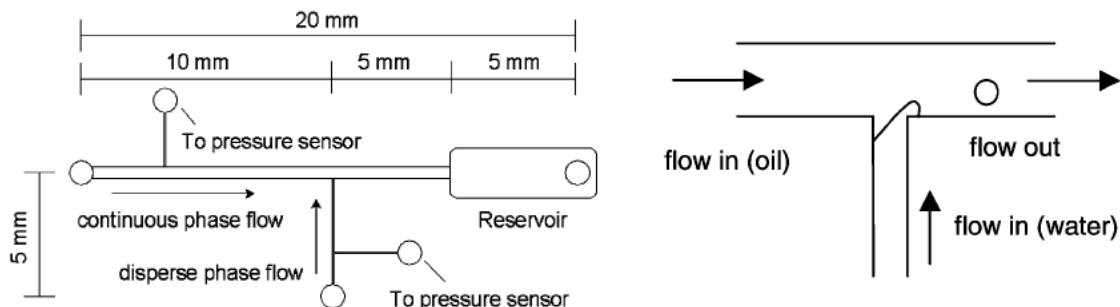


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

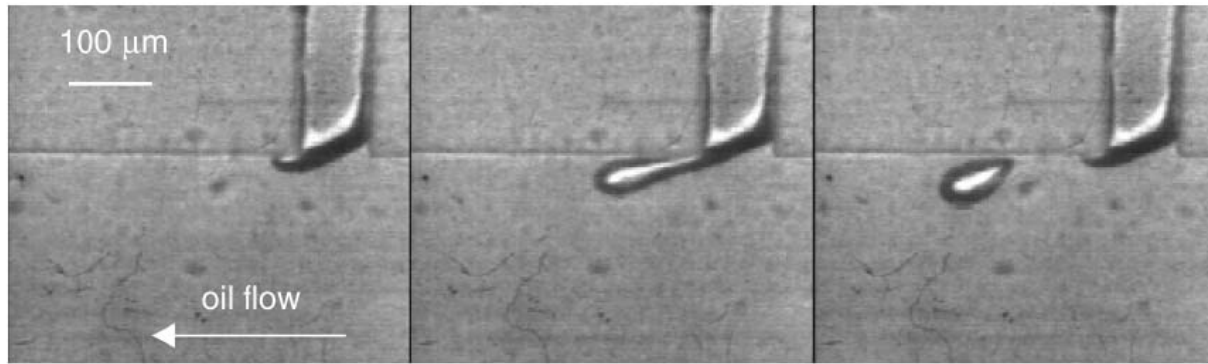


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

(xx) *Claim 58*

3083. Claim 58 of the '091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

3084. Claim 58 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area.”**

3085. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep. For given experimental parameters, regular-sized droplets are reproducibly formed at a uniform speed. The diameter of these droplets is controllable in the range from 100–380 μm as the flow velocity of the continuous phase is varied from 0.01 m s^{-1} to 0.15 m s^{-1} .” Nisisako at Abstract.

3086. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles

and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(e) Invalidity Based on Thorsen

3087. It is my opinion that Thorsen discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

3088. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

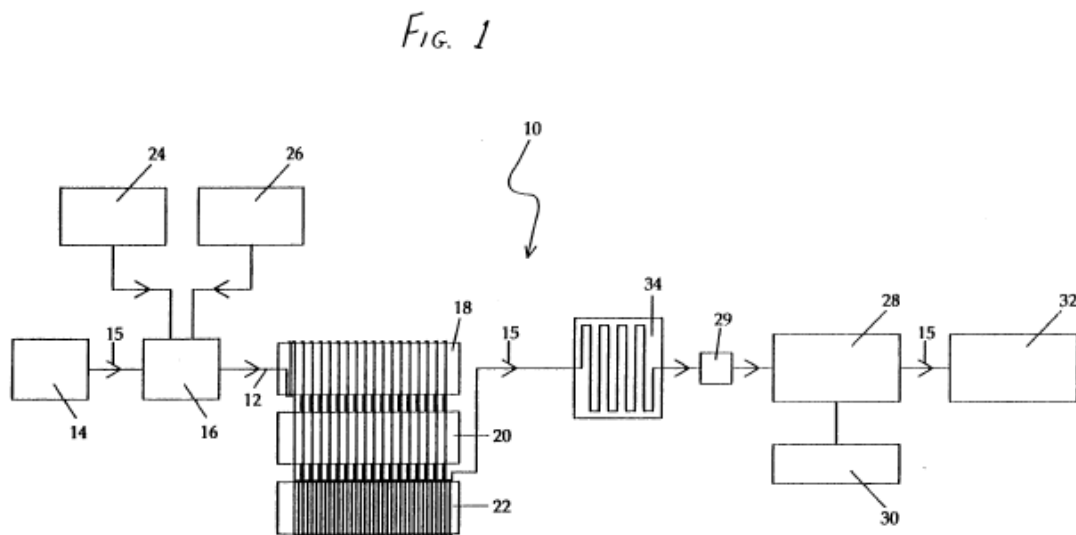
3089. I understand that the Court has not considered whether the preamble of this claim is limiting.

3090. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

3091. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3092. While it is my opinion that Thorsen discloses a method for conducting a reaction

within at least one plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3093. It also would have been obvious to conduct a reaction within at least one plug in

view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3094. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.”

Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3095. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3096. It also would have been obvious to conduct a reaction within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3097. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3098. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the

presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3099. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

3100. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

3101. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing *water and an oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.”

Thorsen at 4163.

3102. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

3103. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3104. Figure 1, showing plug formation, is reproduced below:

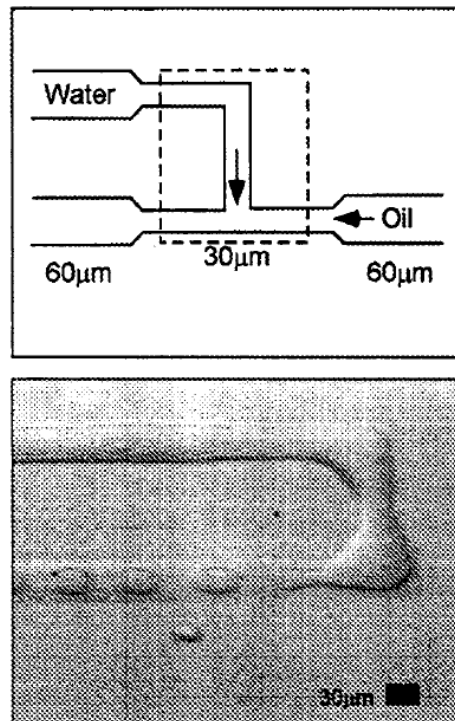


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

3105. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Thorsen with Quake. I incorporate my analysis with respect to ¶¶ 2332-2335, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

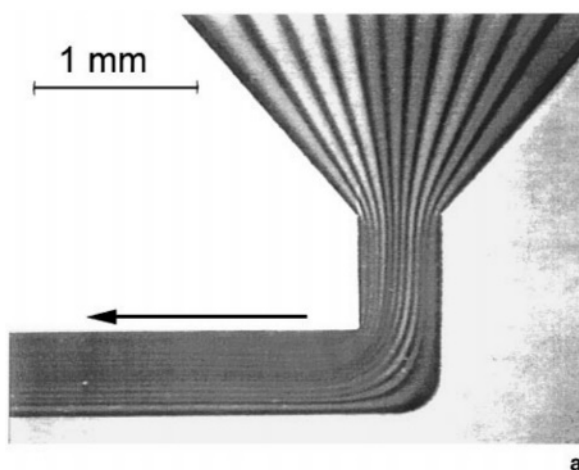
3106. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3107. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, **chemical reactions**, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

3108. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in

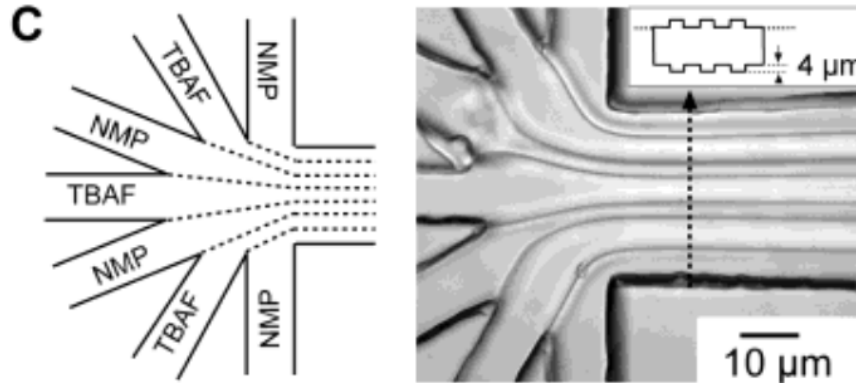
view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3109. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3110. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that

“[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



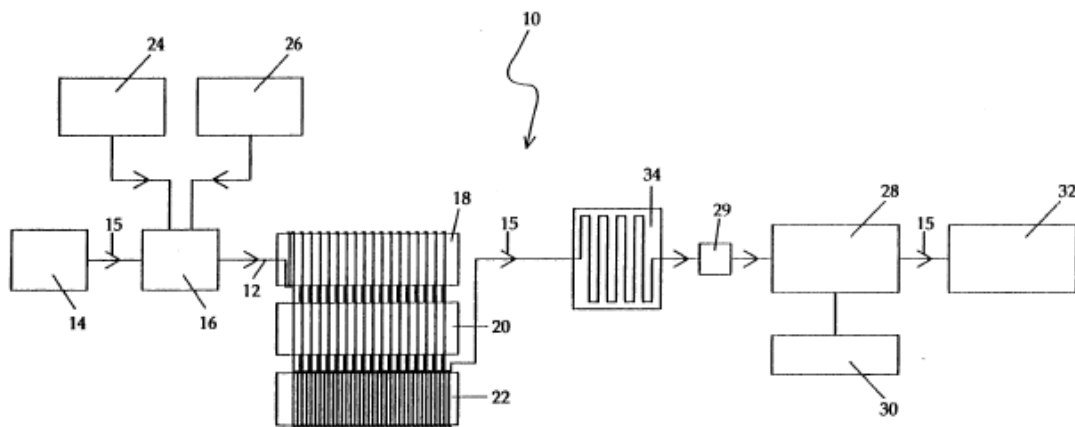
Whitesides at 845-846.

3111. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3112. While it is my opinion that Thorsen discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture

comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3113. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these

assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3114. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagents are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3115. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3116. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3117. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

3118. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from

thermodynamic equilibrium.”).

3119. Figure 1, showing droplets surrounded by the oil, is reproduced below:

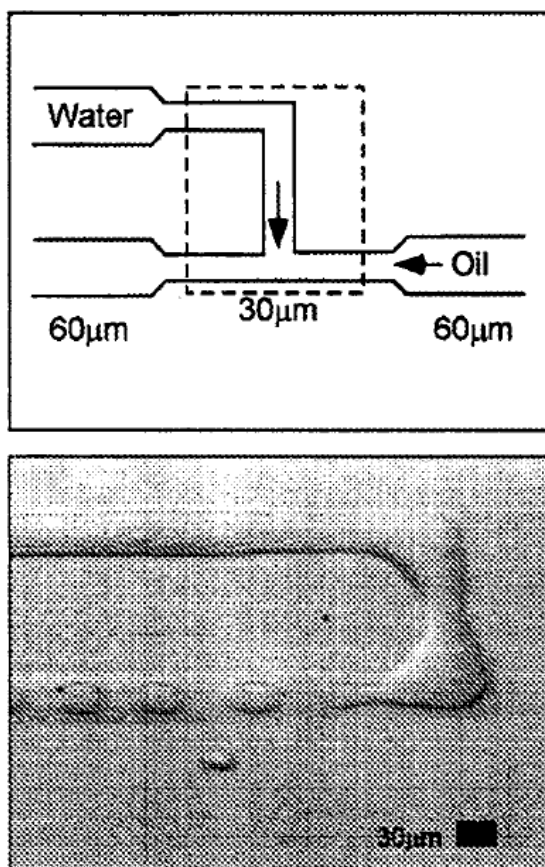


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

(ii) *Claim 2*

3120. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3121. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

3122. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the

presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3123. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

(iii) *Claim 3*

3124. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3125. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

3126. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3127. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing

agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3128. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3129. It also would have been obvious that use a carrier-fluid comprising a fluorinated compound based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

3130. Claim 5 of the ’091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3131. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

3132. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

3133. Thorsen further describes that “[v]arious oils were tested in the device, including decane, tetradecane, and hexadecane, ***combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.***” Thorsen at 4164 (emphasis added).

(v) *Claim 6*

3134. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3135. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

3136. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

(vi) *Claim 11*

3137. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3138. Claim 11 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

3139. Thorsen satisfies this limitation. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3140. It also would have been obvious that the reaction of the plug-fluids forms a

soluble reaction product within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 27*

3141. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3142. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

3143. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163. From Thorsen, a POSA would have known that different oils could be used to form the carrier fluid.

3144. A POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* '091 Patent at Table 1 (refractive index of water is 1.3330) *to* Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

(viii) *Claim 29*

3145. Claim 29 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3146. Claim 29 further recites: “**employing a number of devices in parallel.**”

3147. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2378-2380, demonstrating how Quake discloses employing a number of devices in parallel.

3148. It also would have been obvious to employ a number of devices in parallel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) Claim 31

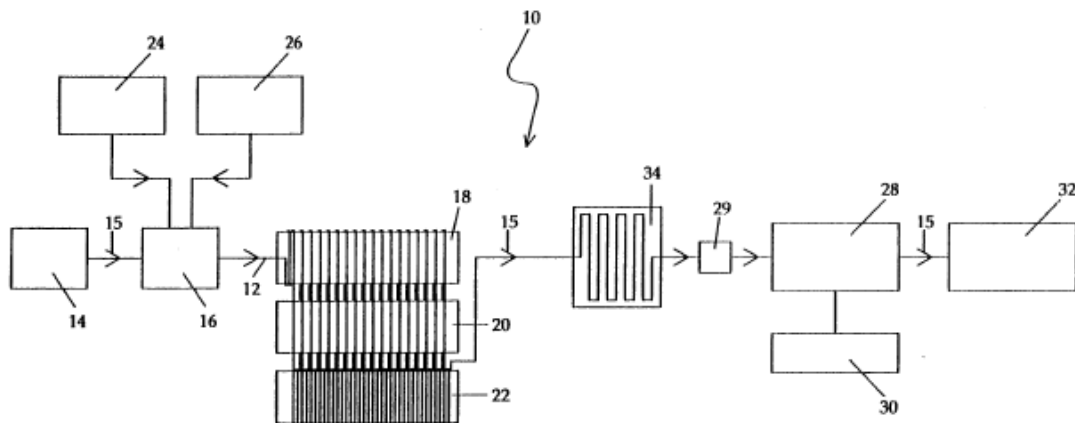
3149. Claim 31 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3150. Claim 31 further recites: “**the reaction is a polymerization reaction.**”

3151. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3152. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3153. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3154. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3155. It also would have been obvious to conduct a polymerization reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

3156. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3157. Claim 33 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.”**

3158. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively. The water partially obstructs flow at the junction, but is not broken off at the channel interface as in tmditional crossflow devices. Droplet formation is achieved by high shear forces genemted at the leading edge of the water perpendicular to the oil flow, genemting picoliter-scale droplets.” Thorsen at 4163.

3159. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

3160. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3161. Claim 35 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

3162. Thorsen satisfies this limitation. For example, Thorsen describes that “[d]roplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating *picoliter-scale* droplets.” Thorsen at 4163 (emphasis added).

(xii) *Claim 36*

3163. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

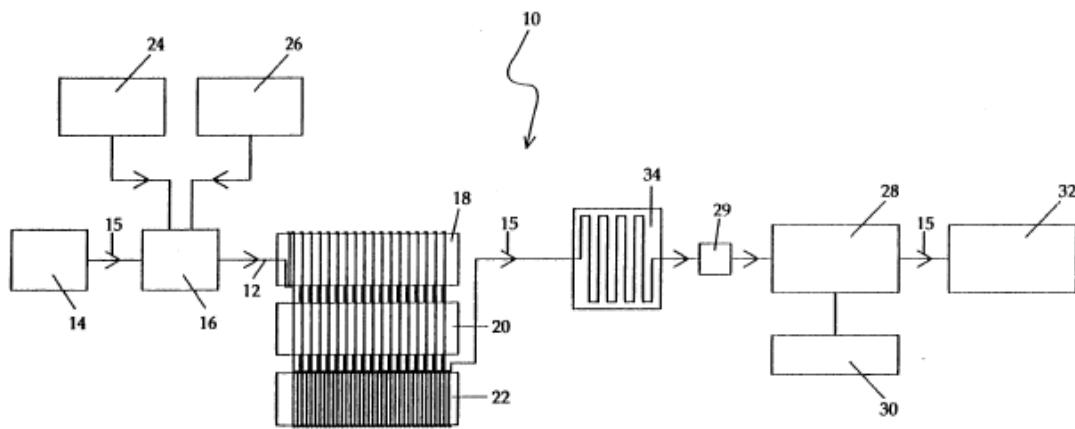
3164. I understand that the Court has not considered whether the preamble of this claim is limiting.

3165. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

3166. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3167. While it is my opinion that Thorsen discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3168. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3169. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3170. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that reactions could be conducted within droplets in a microfluidic system.

3171. It also would have been obvious to conduct a reaction within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3172. Claim 36 further recites: **“introducing a carrier-fluid into a first microchannel of a device.”**

3173. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3174. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

3175. Claim 36 further recites: **“simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first**

microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

3176. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing *water and an oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

3177. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

3178. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3179. Figure 1, showing plug formation, is reproduced below:

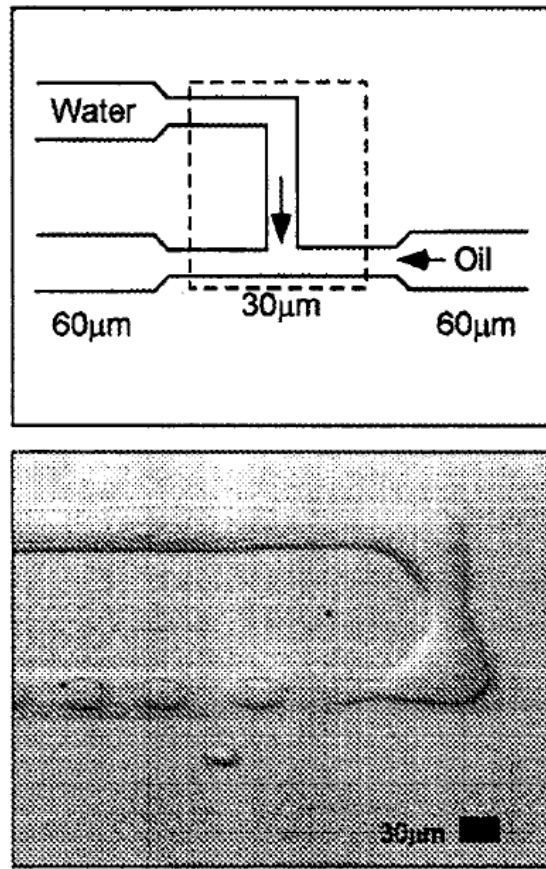


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

3180. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Thorsen with Quake. I incorporate my analysis with respect to ¶¶ 2412-2415, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

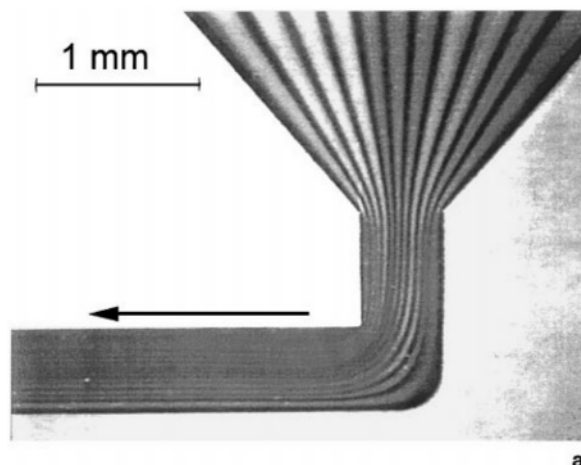
3181. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing

“multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

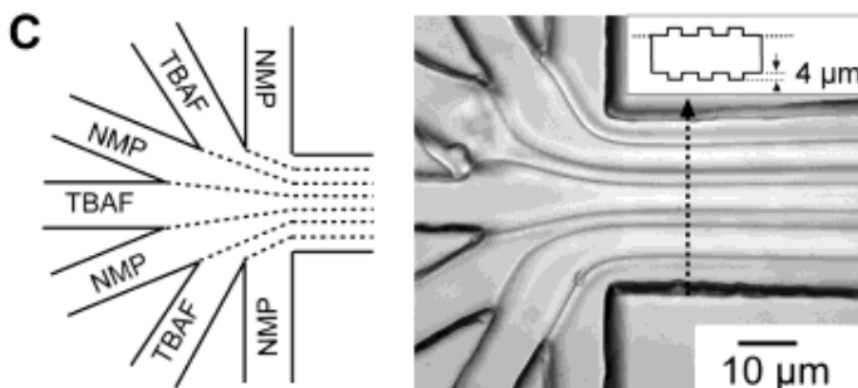
3182. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

3183. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3184. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduces in the same conduit and rapidly mix. Firgure 4a of Erbacher is reproduced below:



3185. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

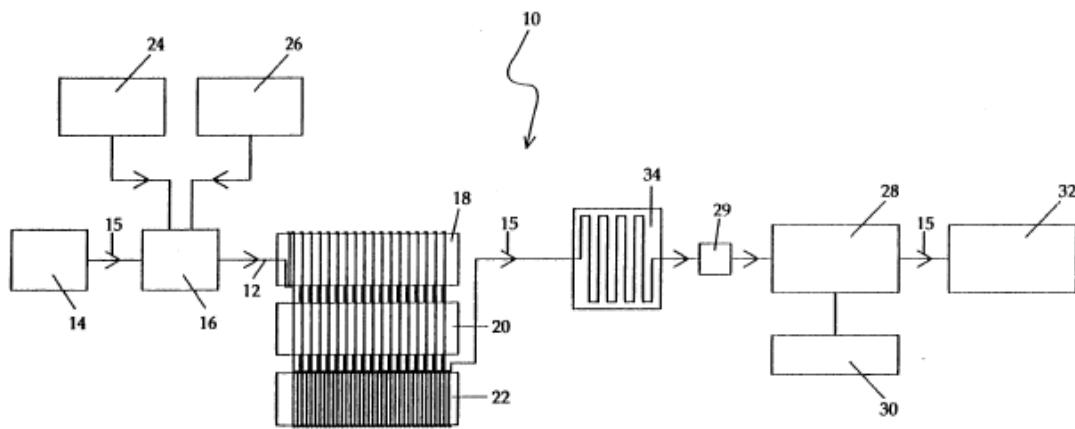


Whitesides at 845-846.

3186. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3187. While it is my opinion that Thorsen discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3188. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3189. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3190. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26.

These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3191. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3192. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

3193. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

3194. Figure 1, showing droplets surrounded by the oil, is reproduced below:

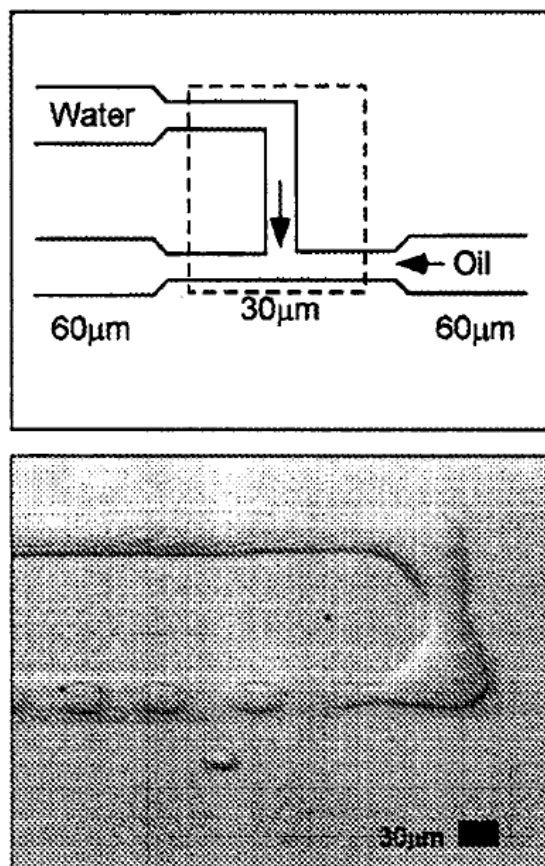


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

(xiii) *Claim 37*

3195. The preamble of claim 37 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3196. I understand that the Court has not considered whether the preamble of this claim is limiting.

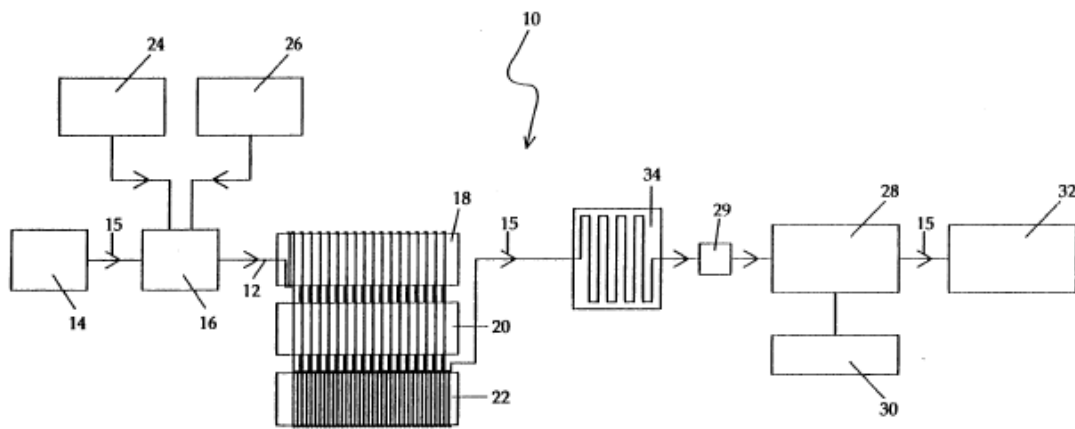
3197. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . .

[W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

3198. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3199. While it is my opinion that Thorsen discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3200. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3201. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3202. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3203. It also would have been obvious to conduct a reaction within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3204. Claim 37 further recites: **“introducing a carrier-fluid into a first microchannel of a device.”**

3205. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3206. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

3207. Claim 37 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first**

microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”

3208. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing *water and an oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

3209. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

3210. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3211. Figure 1, showing plug formation, is reproduced below:

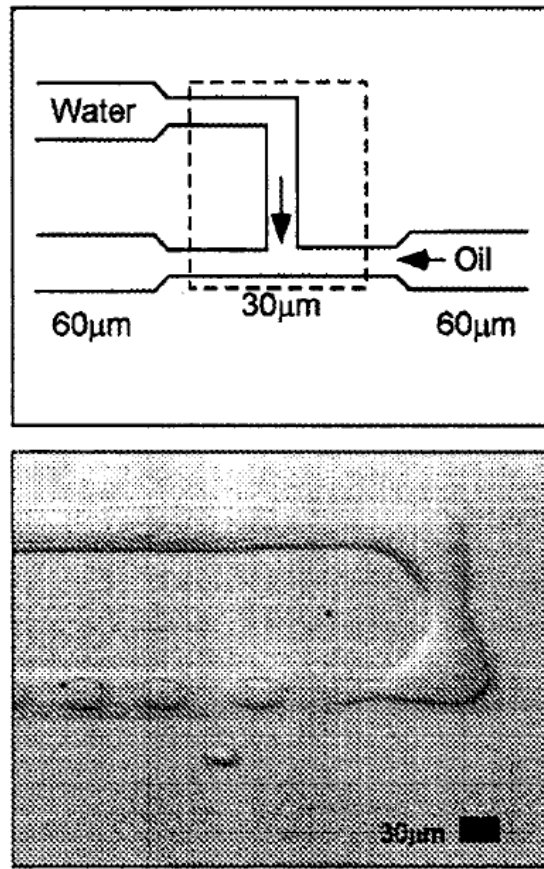


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

3212. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Thorsen with Quake. I incorporate my analysis with respect to ¶¶ 2443-2446, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

3213. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes

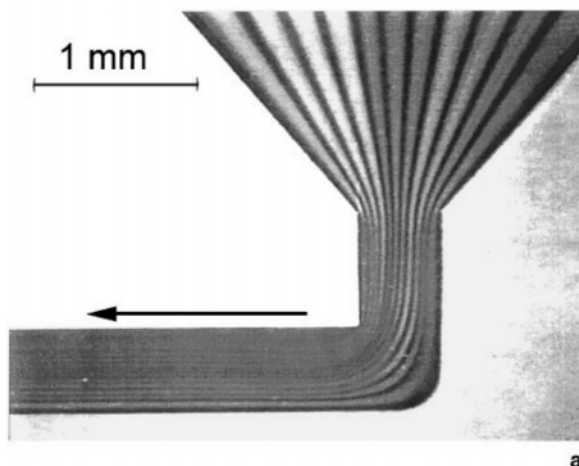
introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3214. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

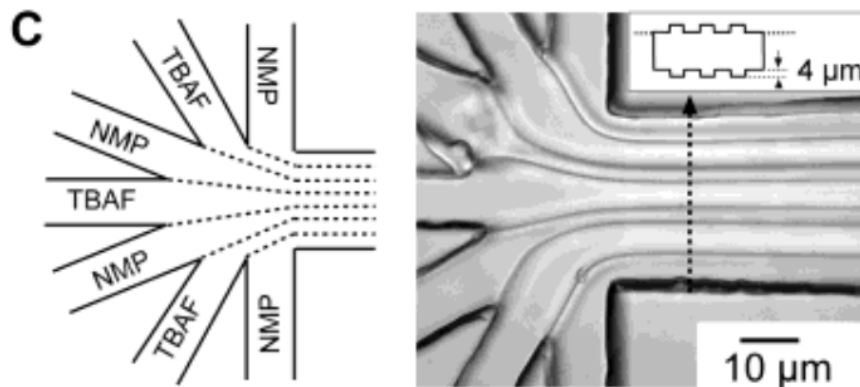
3215. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3216. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two

solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3217. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



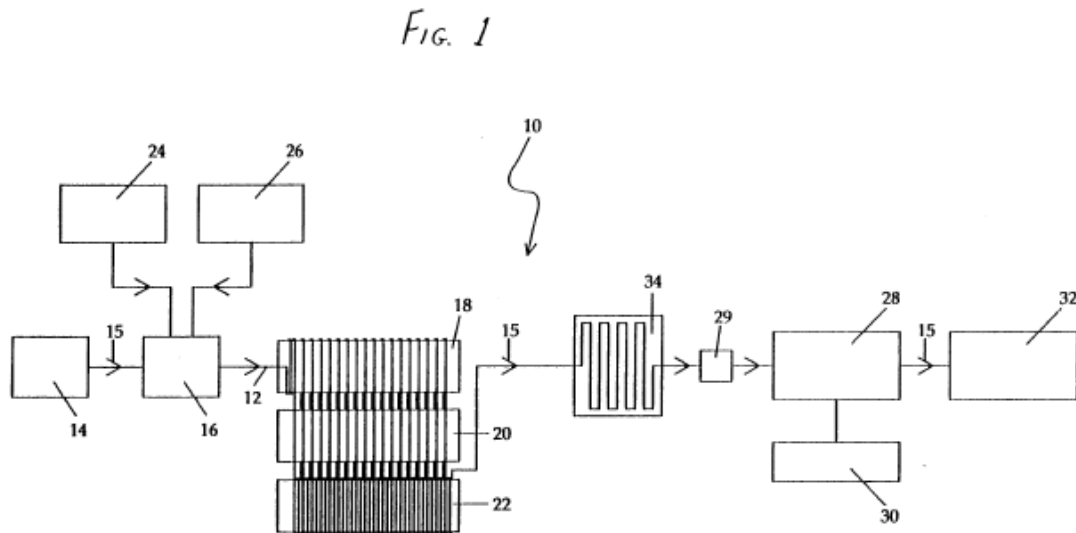
Whitesides at 845-846.

3218. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3219. While it is my opinion that Thorsen discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative

thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3220. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3221. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3222. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for

producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3223. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3224. Claim 37 further recites: “**each plug is substantially surrounded by carrier.**”

3225. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

3226. Figure 1, showing droplets surrounded by the oil, is reproduced below:

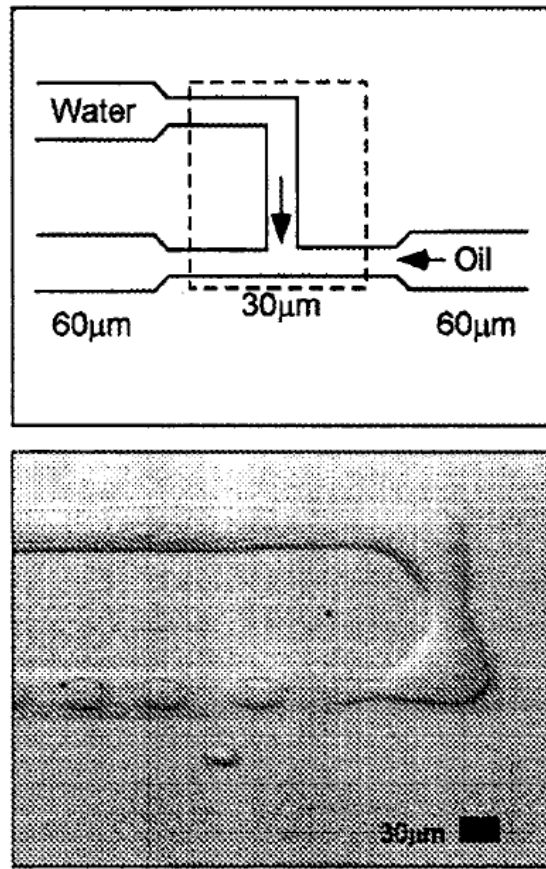


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

(xiv) *Claim 38*

3227. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3228. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

3229. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture,

respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3230. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

(xv) *Claim 39*

3231. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3232. Claim 39 further recites: “**the carrier-fluid comprises at least one surfactant.**”

3233. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, *often in the presence of a surfactant*, to create small droplets.” Thorsen at 4163 (emphasis added).

3234. Thorsen further describes that “[v]arious oils were tested in the device, including decane, tetradecane, and hexadecane, *combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.*” Thorsen at 4164 (emphasis added).

(xvi) *Claim 43*

3235. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3236. Claim 43 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

3237. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art

references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2471-2473, demonstrating how Quake discloses a reaction forming a soluble reaction product within at least one plug.

3238. It also would have been obvious that the reaction of the plug-fluids form a soluble reaction product within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xvii) Claim 53

3239. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3240. Claim 53 further recites: “**employing a number of devices in parallel.**”

3241. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2474-2477, demonstrating how Quake discloses employing a number of devices in parallel.

3242. It also would have been obvious to employ a number of devices in parallel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) Claim 56

3243. Claim 56 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3244. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

3245. Thorsen satisfies this limitation. For example, Thorsen describes that “[d]roplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating *picoliter-scale* droplets.” Thorsen at 4163 (emphasis added).

(xix) Claim 57

3246. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

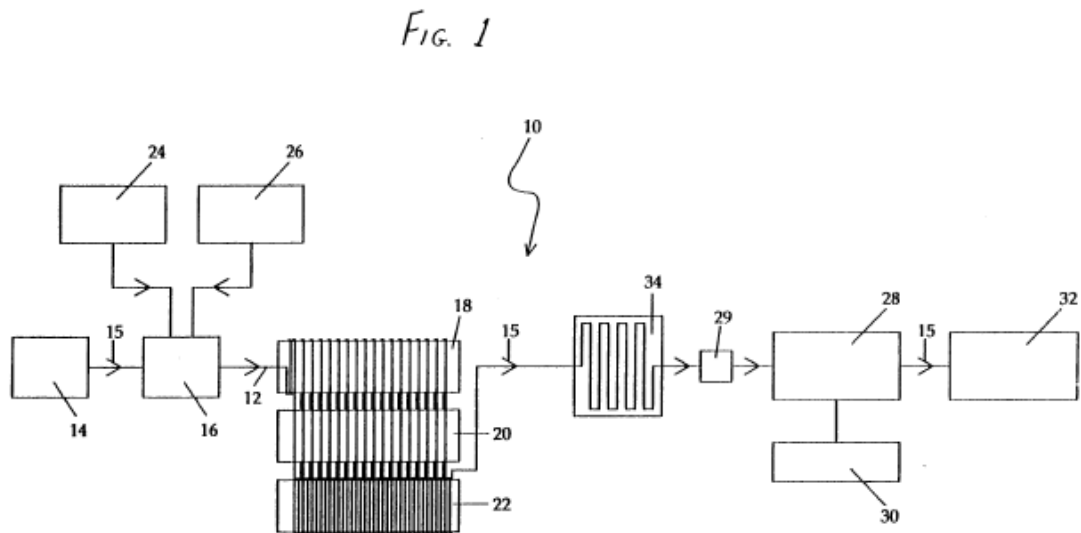
3247. I understand that the Court has not considered whether the preamble of this claim is limiting.

3248. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

3249. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3250. While it is my opinion that Thorsen discloses a method for conducting a reaction

within at least one plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3251. It also would have been obvious to conduct a reaction within at least one plug in

view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3252. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.”

Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3253. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

3254. It also would have been obvious to conduct a reaction within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3255. Claim 57 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3256. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the

presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

3257. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164; *see also* Thorsen at 4165 (“In the microfluidic device, a shear gradient is established as water tries to expand into the pressurized continuous phase.”).

3258. Claim 57 further recites: “**introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

3259. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing *water and an oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the

leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

3260. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

3261. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

3262. Figure 1, showing plug formation, is reproduced below:

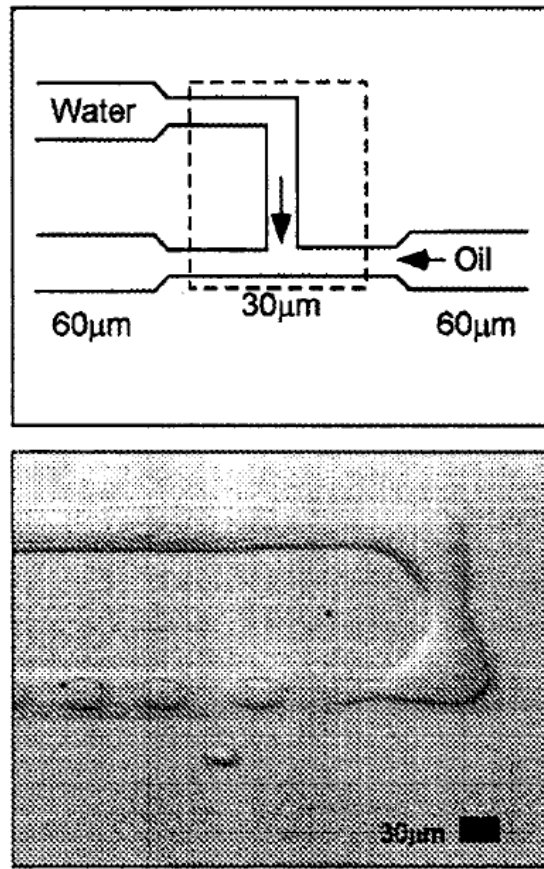


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

3263. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Thorsen with Quake. I incorporate my analysis with respect to ¶¶ 2497-2500, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

3264. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes

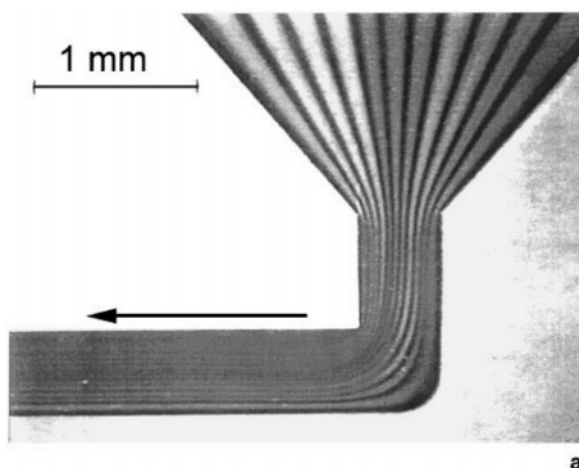
introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3265. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

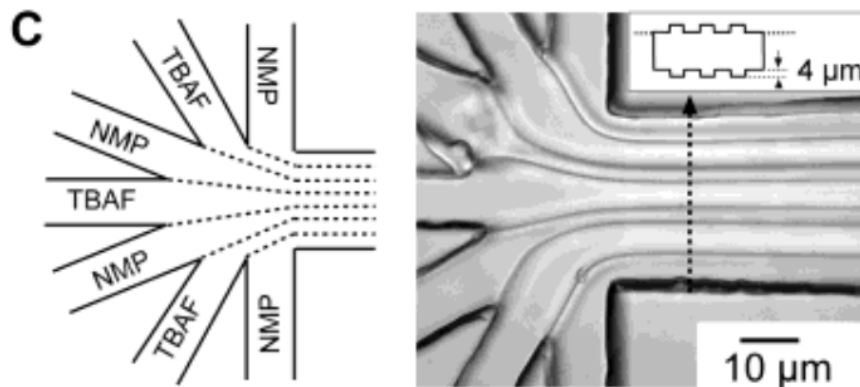
3266. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3267. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two

solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3268. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.



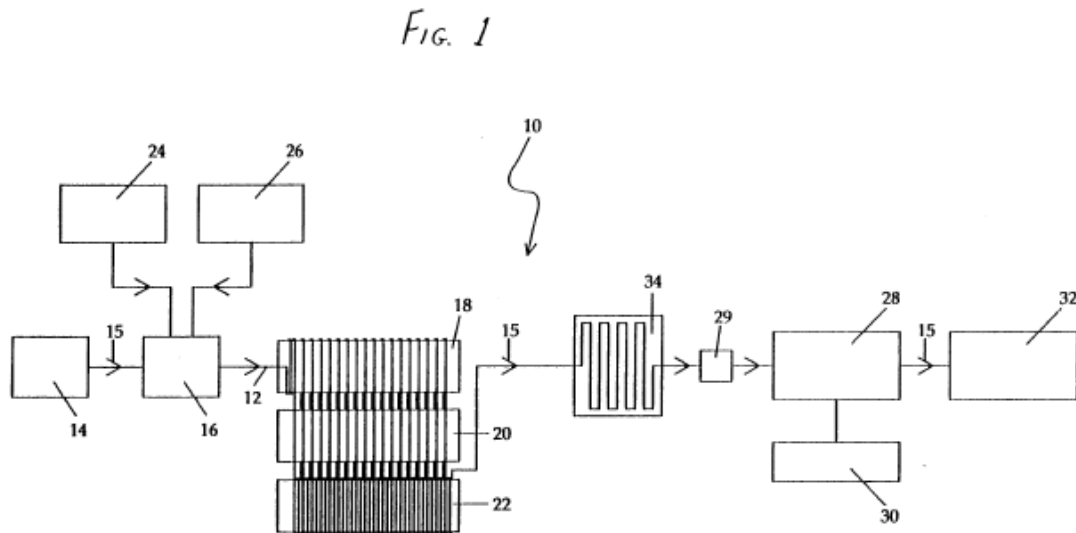
Whitesides at 845-846.

3269. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3270. While it is my opinion that Thorsen discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative

thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3271. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3272. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3273. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for

producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3274. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3275. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

3276. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

3277. Figure 1, showing droplets surrounded by the oil, is reproduced below:

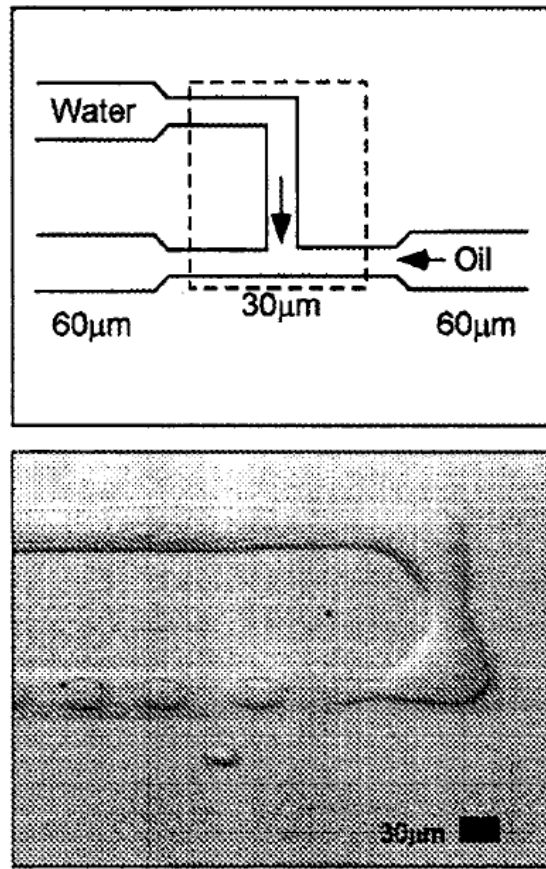


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

(xx) Claim 58

3278. Claim 58 of the '091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

3279. Claim 58 further recites: “**each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area.**”

3280. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively. The water partially obstructs flow at the junction, but is

not broken off at the channel interface as in traditional crossflow devices. Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

3281. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(f) Invalidity Based on Seki

3282. It is my opinion that Seki discloses and/or renders obvious all elements of claims 1-3, 5-6, 11, 27, 29, 31, 33, 35-39, 43, 53, and 56-58 of the '091 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

3283. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3284. I understand that the Court has not considered whether the preamble of this claim is limiting.

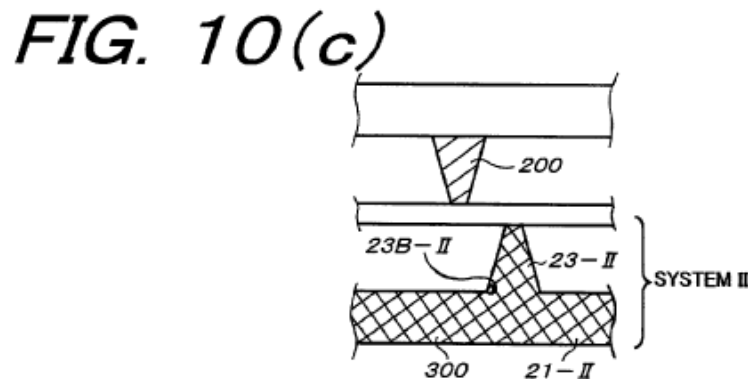
3285. Regardless of whether the preamble is limiting, Seki satisfies this claim limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third

flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3286. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:

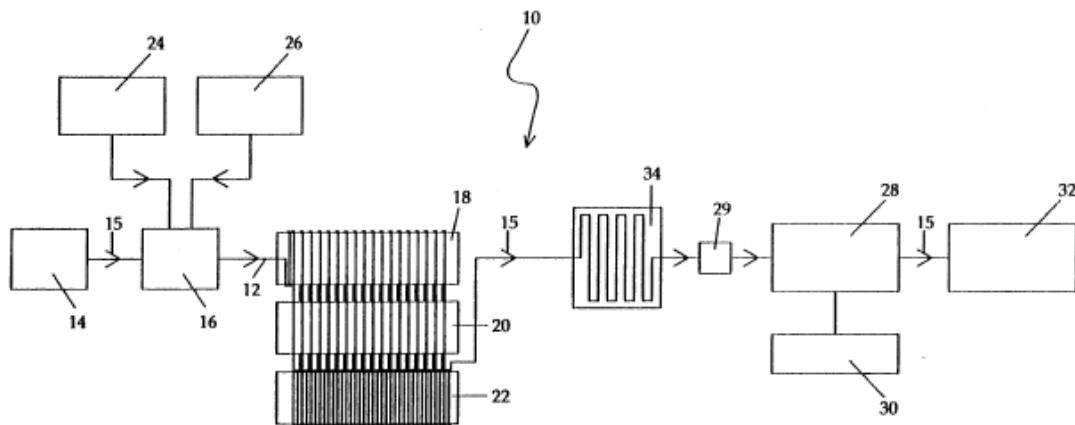


Seki at Fig. 10(c).

3287. While it is my opinion that Seki discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using

the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3288. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in

an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3289. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns

(1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3290. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3291. It also would have been obvious to conduct a reaction within at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3292. Claim 1 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3293. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that

of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3294. Claim 1 further recites: “**simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.**”

3295. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through

an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

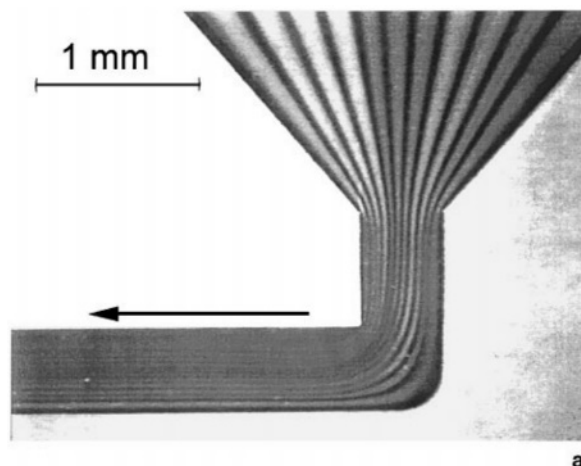
3296. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Seki with Quake. I incorporate my analysis with respect to ¶¶ 2332-2335, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

3297. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3298. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, **chemical reactions**, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

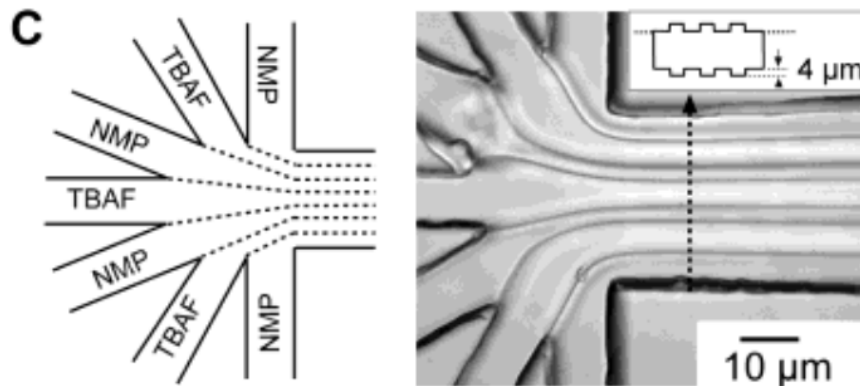
3299. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3300. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3301. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that

“[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

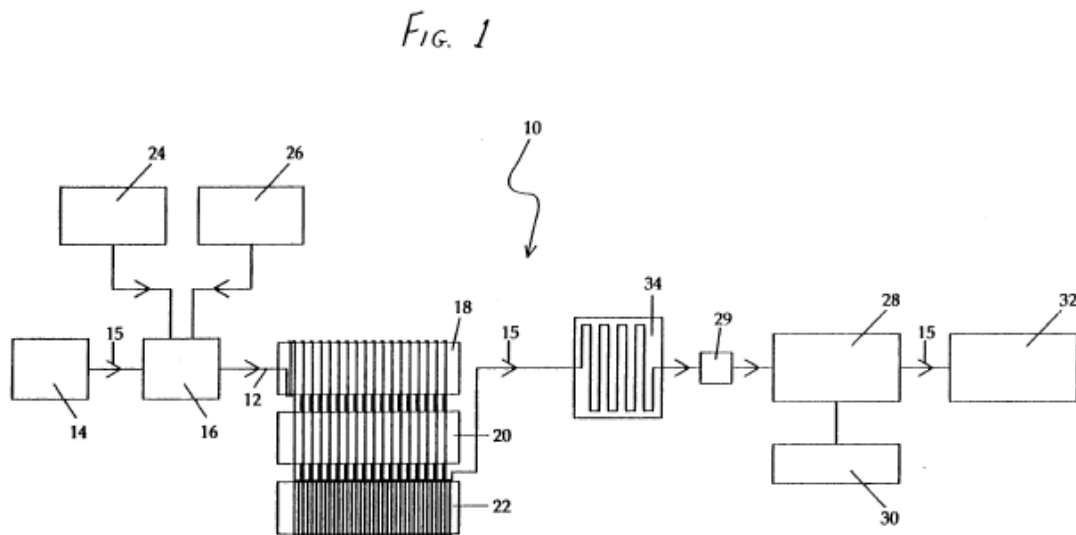


Whitesides at 845-846.

3302. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3303. While it is my opinion that Seki discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the

carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3304. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at

Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3305. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA

would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3306. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3307. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3308. Claim 1 further recites: “**each plug is substantially surrounded by carrier.**”

3309. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel

to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

(ii) *Claim 2*

3310. Claim 2 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3311. Claim 2 further recites: “**the carrier-fluid comprises an oil.**”

3312. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising an oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3313. It also would have been obvious to use a carrier-fluid comprising an oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3314. It also would have been obvious to use a carrier-fluid comprising an oil in view of

Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3315. It also would have been obvious to use a carrier-fluid comprising an oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 3*

3316. Claim 3 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3317. Claim 3 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

3318. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . .

offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3319. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3320. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3321. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 5*

3322. Claim 5 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3323. Claim 5 further recites: “**the carrier-fluid comprises at least one surfactant.**”

3324. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3325. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3326. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where

biocompatibility is required.” *Id.* at 6:46-50.

3327. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 6*

3328. Claim 6 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3329. Claim 6 further recites: “**at least one of the plug-fluids comprises a solvent.**”

3330. Seki satisfies this limitation. For example, Seki describes a reaction using glucose as a solvent: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

(vi) *Claim 11*

3331. Claim 11 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3332. Claim 11 further recites: “**the reaction of the plug-fluids forms a soluble reaction product within at least one plug.**”

3333. Seki satisfies this limitation. For example, Seki describes a soluble glucose reaction product: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing

glucose.” Seki at [0139].

(vii) *Claim 27*

3334. Claim 27 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3335. Claim 27 further recites: “**refractive indices of the carrier-fluid and the plug-fluids are substantially similar.**”

3336. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2568-2571, demonstrating how Quake discloses that the refractive indices of the carrier-fluid and the plug-fluids are substantially similar.

3337. For example, a POSA would have known that the refractive index of, for example, silicone oil was similar to that of water. *Compare* '091 Patent at Table 1 (refractive index of water is 1.3330) *to* Gelest at 2 (stating that the “Refractive Index” of silicone fluids is between “1.393-1.403.”).

3338. It also would have been obvious to use a carrier-fluid and plug-fluids with substantially similar refractive indices based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(viii) *Claim 29*

3339. Claim 29 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3340. Claim 29 further recites: “**employing a number of devices in parallel.**”

3341. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2378-2380, demonstrating how Quake discloses employing a number of devices in parallel.

3342. It also would have been obvious to employ a number of devices in parallel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 31*

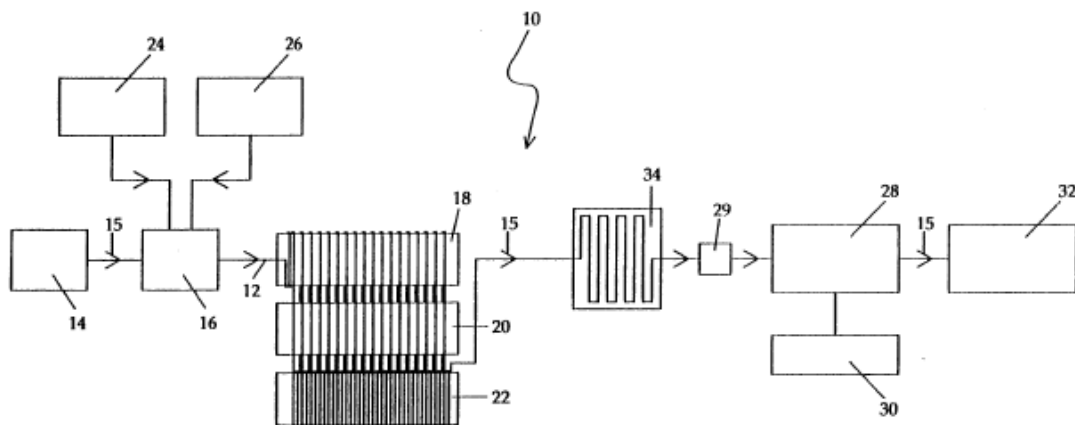
3343. Claim 31 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3344. Claim 31 further recites: “**the reaction is a polymerization reaction.**”

3345. I understand that Bio-Rad is contending that “PCR is a polymerization reaction.” *See* Appendix A to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 67. Under Plaintiffs’ interpretation of the term, Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.”

Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3346. It also would have been obvious to conduct a polymerization reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For

example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3347. It also would have been obvious to conduct a polymerization reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A)

and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3348. It also would have been obvious to conduct a polymerization reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 33*

3349. Claim 33 of the '091 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3350. Claim 33 further recites: **“each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet.”**

3351. Seki satisfies this limitation. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025].

3352. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 35*

3353. Claim 35 of the '091 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

3354. Claim 35 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

3355. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025]. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2393-2397, demonstrating how Quake discloses that the volume of at least one plug is about 1 femtoliter to about 250 nL.

3356. It also would have been obvious that the volume of at least one plug is about 1 femtoliter to about 250 nL based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 36*

3357. The preamble of claim 1 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3358. I understand that the Court has not considered whether the preamble of this claim is limiting.

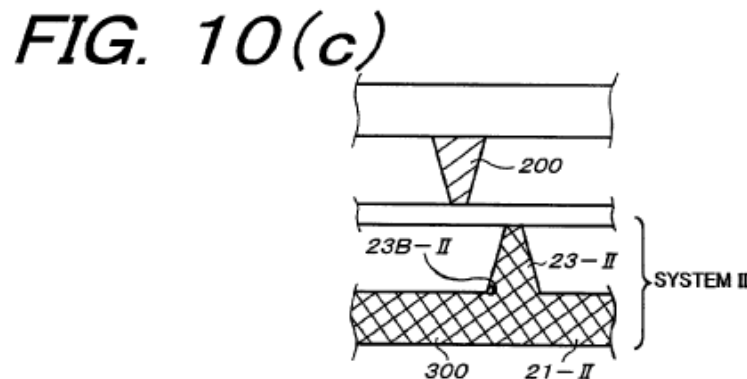
3359. Regardless of whether the preamble is limiting, Seki satisfies this claim limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by

only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3360. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:

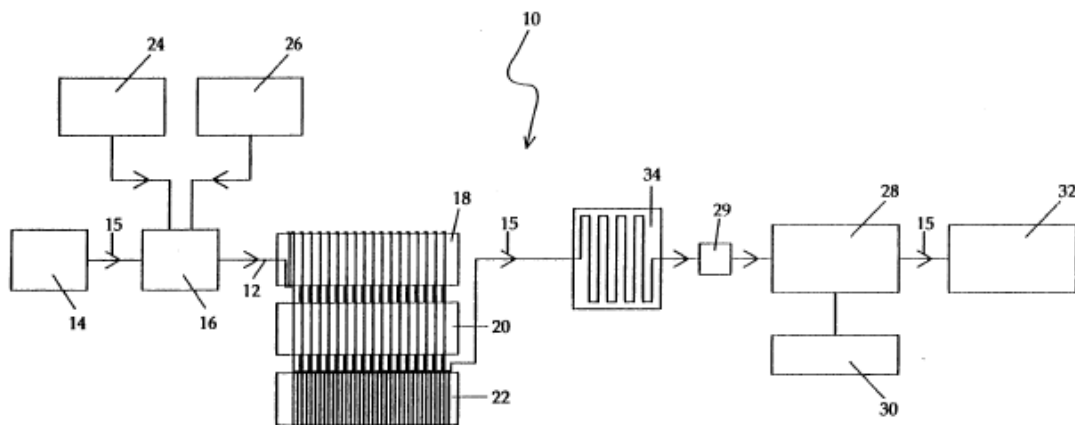


Seki at Fig. 10(c).

3361. While it is my opinion that Seki discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Seki with

one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3362. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate.

For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3363. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports

(Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3364. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3365. It also would have been obvious to conduct a reaction within at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3366. Claim 36 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3367. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a

second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3368. Claim 36 further recites: **“simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier fluid at a junction of the first inlet and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

3369. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first

flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

3370. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Seki with Quake. I incorporate my analysis with respect to ¶¶ 2412-2415, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

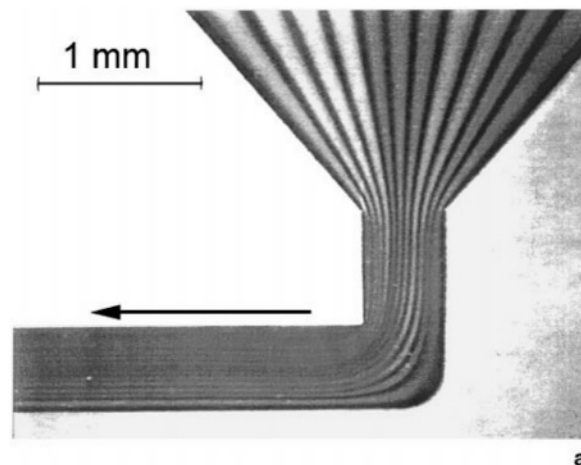
3371. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3372. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of

time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

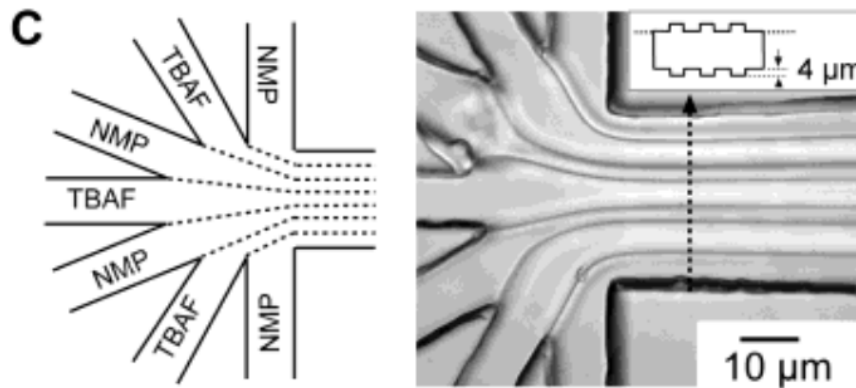
3373. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3374. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3375. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could

be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

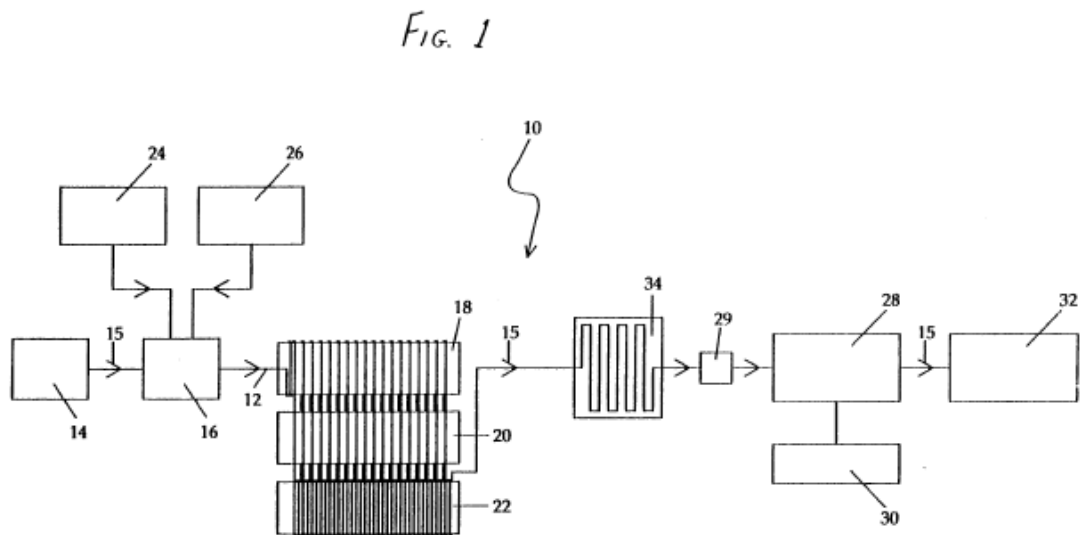


Whitesides at 845-846.

3376. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into a first inlet in fluid communication with a microchannel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3377. While it is my opinion that Seki discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a

stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3378. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE)

analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3379. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR

amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3380. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

3381. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3382. Claim 36 further recites: “**each plug is substantially surrounded by carrier.**”

3383. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and

the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

(xiii) *Claim 37*

3384. The preamble of claim 37 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

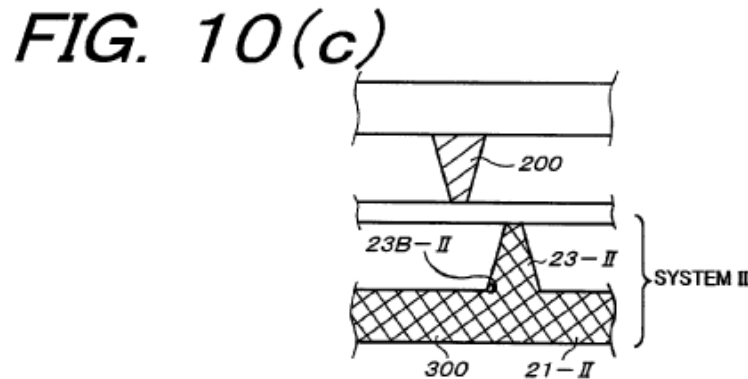
3385. I understand that the Court has not considered whether the preamble of this claim is limiting.

3386. Regardless of whether the preamble is limiting, Seki satisfies this claim limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

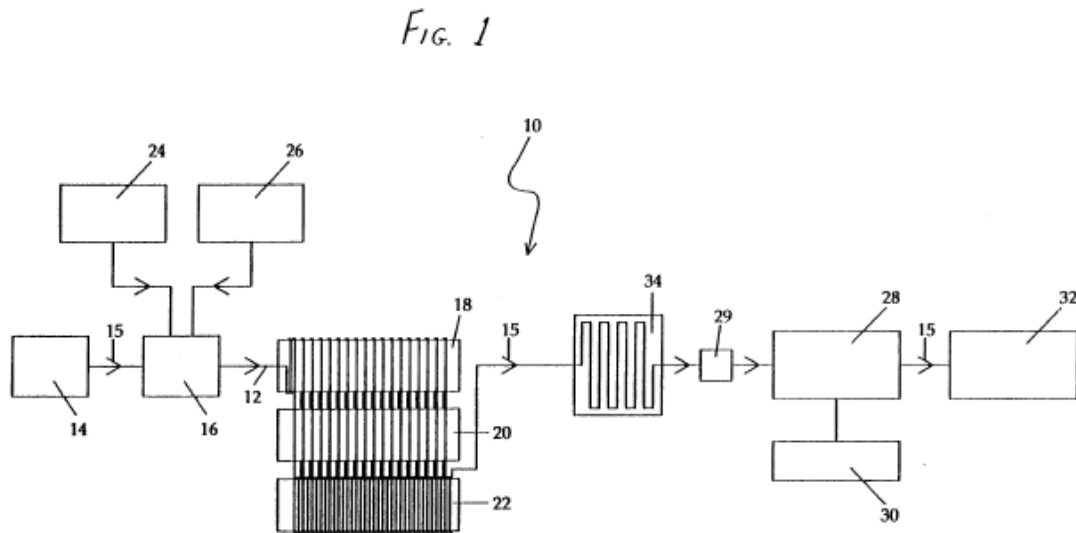
3387. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

3388. While it is my opinion that Seki discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small

volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3389. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from

the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3390. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3391. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may

react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that PCR reactions could be conducted within droplets in a microfluidic system.

3392. It also would have been obvious to conduct a reaction within at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3393. Claim 37 further recites: **“introducing a carrier-fluid into a first microchannel of a device.”**

3394. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3395. Claim 37 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

3396. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

3397. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Seki with Quake. I incorporate my analysis with respect to ¶¶ 2443-2446, demonstrating how Quake discloses

different droplet-formation configurations for a microfluidic device.

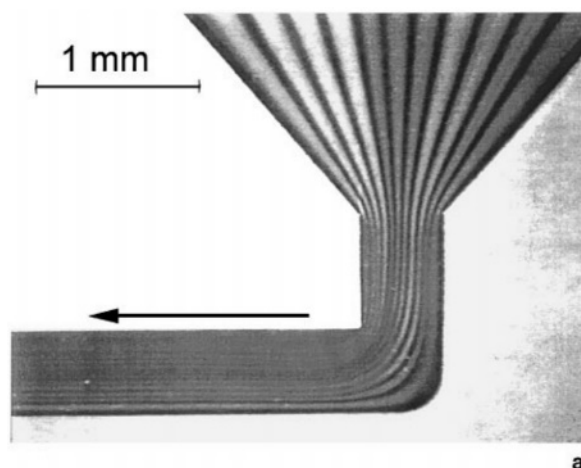
3398. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3399. It also would been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346 (emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

3400. It also would been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed

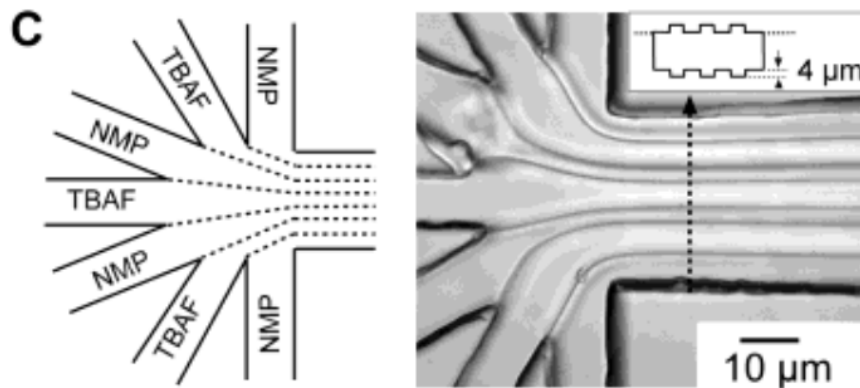
are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3401. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3402. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures”

such as those shown in Fig. 4C, reproduced below.

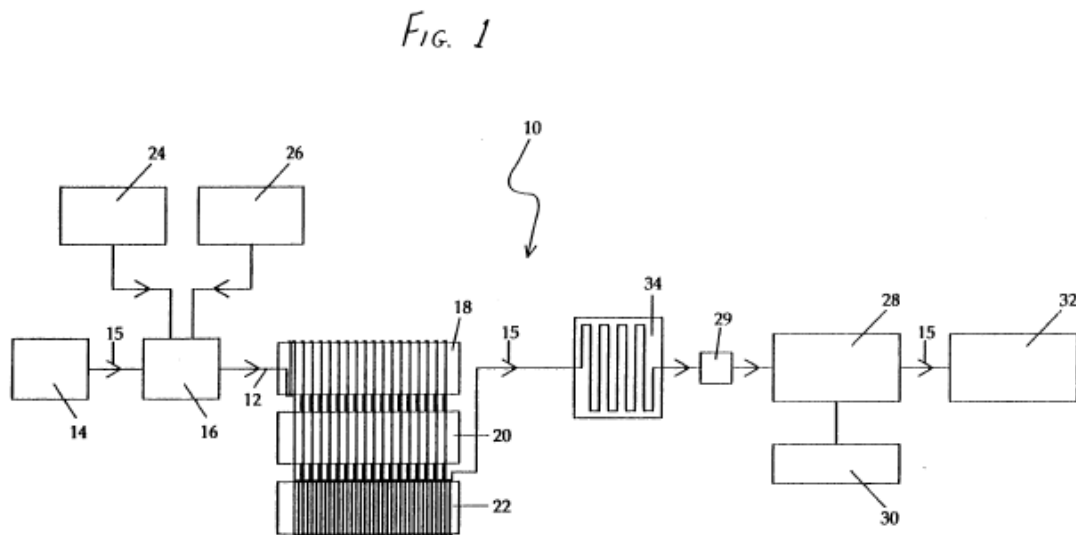


Whitesides at 845-846.

3403. It also would have been obvious simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3404. While it is my opinion that Seki discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an

enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3405. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally,

“[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3406. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3407. It also would have been obvious that each plug comprises reagents so that a

reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3408. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3409. Claim 37 further recites: “**each plug is substantially surrounded by carrier.**”

3410. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained

in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

(xiv) *Claim 38*

3411. Claim 38 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3412. Claim 38 further recites: “**the carrier-fluid comprises an oil.**”

3413. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2460-2464, demonstrating how Quake discloses an immiscible carrier fluid that is an oil.

3414. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use an oil with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3415. It also would have been obvious to use an oil oil in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3416. It also would have been obvious to use an oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3417. It also would have been obvious to use an oil based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xv) *Claim 39*

3418. Claim 39 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3419. Claim 39 further recites: “**the carrier-fluid comprises at least one surfactant.**”

3420. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3421. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3422. It also would have been obvious to use a carrier-fluid comprising a surfactant in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3423. It also would have been obvious to use a carrier-fluid comprising a surfactant based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xvi) *Claim 43*

3424. Claim 43 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3425. Claim 43 further recites: **“the reaction of the plug-fluids forms a soluble**

reaction product within at least one plug.”

3426. Seki satisfies this limitation. For example, Seki describes a soluble glucose reaction product: “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplets in the reagent 300 for analyzing glucose arises, so that the ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139].

(xvii) Claim 53

3427. Claim 53 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3428. Claim 53 further recites: “**employing a number of devices in parallel.**”

3429. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2474-2477, demonstrating how Quake discloses employing a number of devices in parallel.

3430. It also would have been obvious to employ a number of devices in parallel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xviii) Claim 56

3431. Claim 56 of the '091 patent is dependent on claim 37. I incorporate by reference my analysis with respect to claim 37.

3432. Claim 56 further recites: “**the volume of at least one plug is about 1 femtoliter to about 250 nL.**”

3433. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025]. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 2478-2482, demonstrating how Quake discloses that the volume of at least one plug is about 1 femtoliter to about 250 nL.

3434. It also would have been obvious that the volume of at least one plug is about 1 femtoliter to about 250 nL based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xix) Claim 57

3435. The preamble of claim 57 of the '091 patent recites: “**A method of conducting a reaction within at least one plug.**”

3436. I understand that the Court has not considered whether the preamble of this claim is limiting.

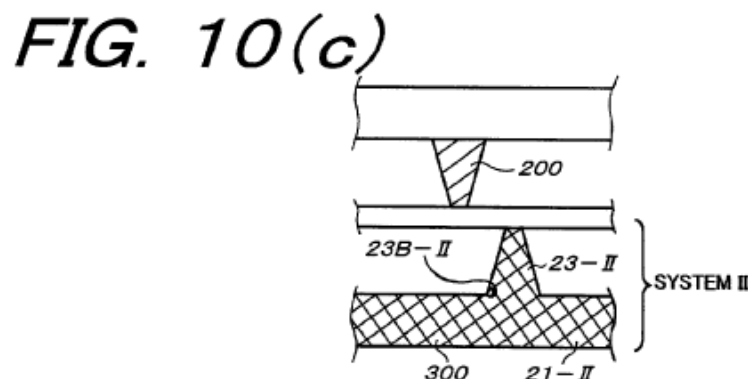
3437. Regardless of whether the preamble is limiting, Seki satisfies this claim limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a

second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3438. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:

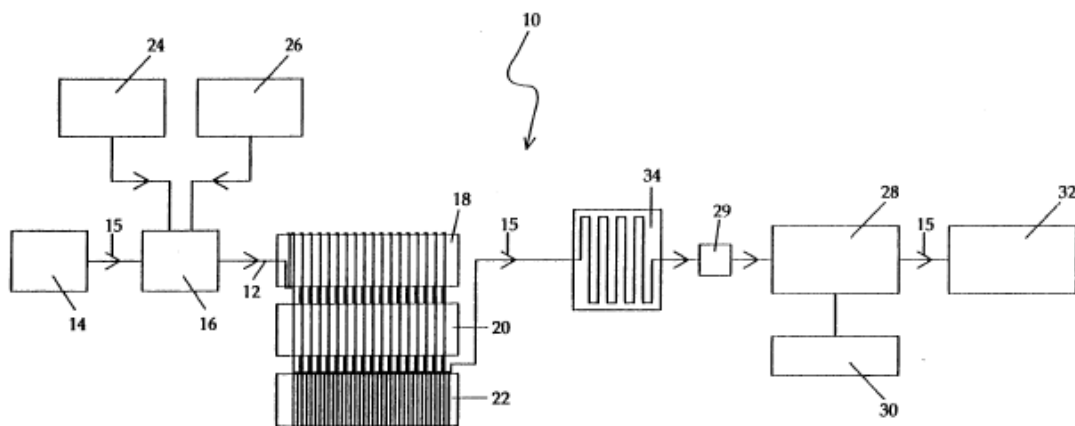


Seki at Fig. 10(c).

3439. While it is my opinion that Seki discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an

apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3440. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template

molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3441. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor

where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3442. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that reactions could be conducted within droplets in a microfluidic system.

3443. It also would have been obvious to conduct a reaction within at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3444. Claim 57 further recites: “**introducing a carrier-fluid into a first microchannel of a device.**”

3445. Seki discloses this limitation. For example, Seki describes a microfluidic system in which a carrier fluid is flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third

flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

3446. Claim 57 further recites: **“introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug.”**

3447. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first

flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

3448. Different droplet-formation configurations were also described in the prior art. For example, it would have been obvious to combine the teachings of Seki with Quake. I incorporate my analysis with respect to ¶¶ 2497-2500, demonstrating how Quake discloses different droplet-formation configurations for a microfluidic device.

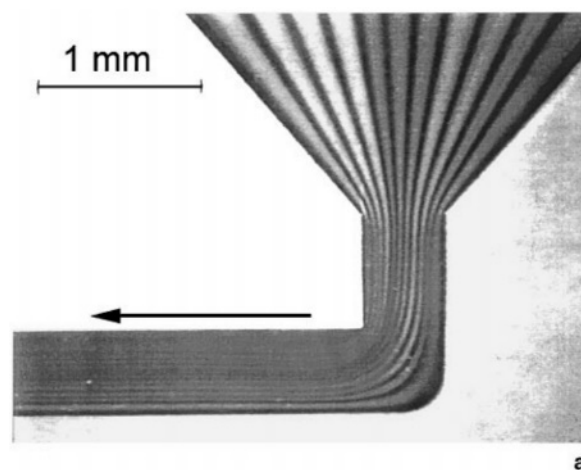
3449. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kenis. Kenis describes introducing “multiple liquid streams flowing laminarly” in a single channel. Kenis at 83. The liquid stream are “reactive species” and reaction occurs at the interface of the laminar streams. Kenis at 83.

3450. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Weigl. Weigl describes a microfluidic device, a so-called “T-sensor,” in which “a sample solution, a receptor (indicator) solutions, and a reference solution are introduced in a common channel.” Weigl at 346. Within these microfluidic devices, “[t]he fluids interact during parallel flow until they exit the microstructure,” and “[a] true μ TAS will perform all analytical functions including smaplying, sample pre-treatment, separation, dilution, mixing steps, *chemical reactions*, and detection in an integrated microfluidic circuit.” Weigl at 346

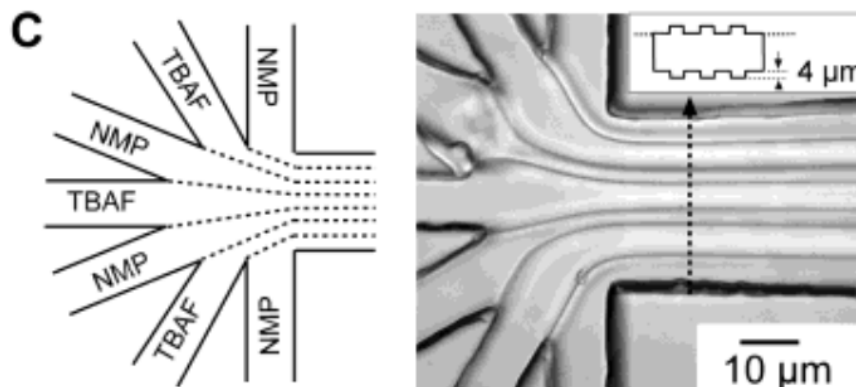
(emphasis added). Using this system, the kinetics of the reaction can be measured “not as a function of time, but of distance from the starting point of the diffusion interaction.” Weigl at 347.

3451. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Kopf-Sill. Kopf-Sill describes a microfluidic device with channels of varied depth which allow for improved mixing. Kopf-Sill, at Abstract. The materials to be mixed are introduced in parallel through two channels that intersect a main channel. Kopf-Sill at 14:3-16.

3452. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Erbacher. Erbacher describes “a device for rapid mixing of two solutions” in which complete mixing of the reactants occurred within just a few seconds. Erbacher at 19. The fluids to be mixed are introduced in the same conduit and rapidly mix. Figure 4a of Erbacher is reproduced below:



3453. It also would be obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel in view of Whitesides. Whitesides describes a similar continuous flow microchannel reactor could be used for various fabrication reactions. Whitesides at 844-845. Whitesides concludes that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below.

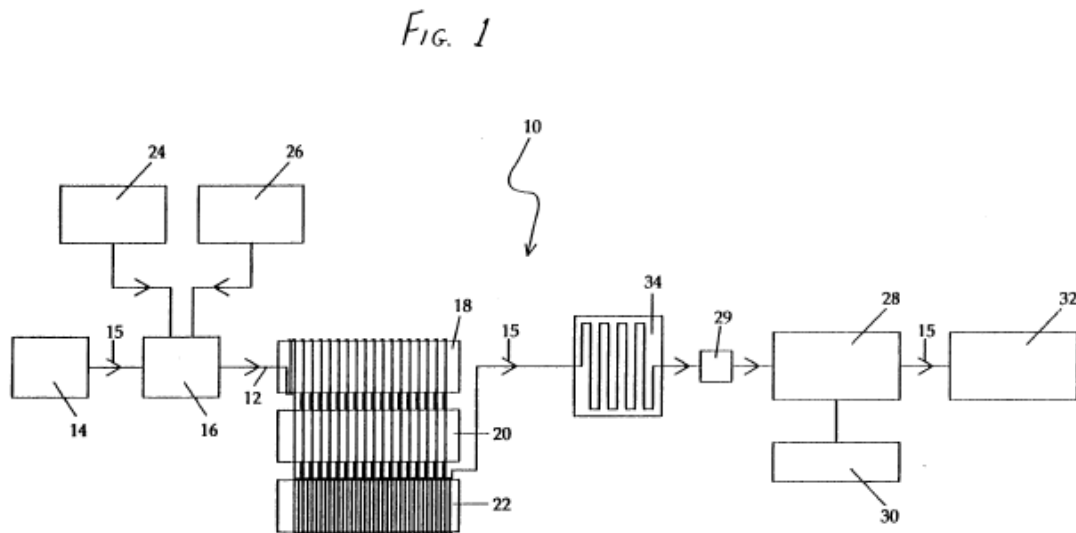


Whitesides at 845.

3454. It also would have been obvious to simultaneously introduce two streams of plug-fluids, each comprising a reagent, into first and second inlets in fluid communication with a microchannel based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.,* Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3455. While it is my opinion that Seki discloses that each plug comprises reagents so that a reaction substantially occurs in the plug, it also would have been obvious to combine the

teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3456. It also would have been obvious that each plug comprises reagents so that a

reaction substantially occurs in the plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3457. It also would have been that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed

channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3458. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3459. It also would have been obvious that each plug comprises reagents so that a reaction substantially occurs in the plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3460. Claim 57 further recites: “**each plug is substantially surrounded by carrier.**”

Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow

channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

(xx) *Claim 58*

3461. Claim 58 of the '091 patent is dependent on claim 57. I incorporate by reference my analysis with respect to claim 57.

3462. Claim 58 further recites: “**each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area.**”

3463. Seki satisfies this limitation. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025].

3464. It also would have been obvious that each plug initially has a cross section that is substantially the same size as the cross section of the channel at the inlet based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2. *Motivation to Combine and Reasonable Expectation of Success*

3465. A POSA would have seen compelling reasons to modify the microfluidic droplet

reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct different types of reactions (including PCR) in small volumes as taught by Corbett, Lagally, Burns (1996), or Wang. This is because the prior art clearly taught that reactions could be conducted within microfluidic droplets, and there were numerous advantages associated with these microfluidic droplet reactors. In particular, a POSA would have considered it obvious to modify the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR based on numerous teachings in the art, including Corbett, Lagally, and Burns (1996), which discussed small-scale and even on-chip PCR (*see, e.g.*, Burns (1996)). A POSA would have had a reasonable expectation of success in so modifying, as evidenced by both the prior art and contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. Indeed, Quake itself describes both enzymatic reactions with biological molecules and PCR within microfluidic droplets. Quake at [0080] and [0170].

3466. A POSA would have been strongly motivated to perform reactions, including PCR, in the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki because doing so would have provided the substantial benefits known to be associated with microfluidic reactors. For example, Nisisako noted that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation, and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” *Treating liquid samples in droplet shape has the advantage that dead volume can be decreased.* Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is *likely to become increasingly important.*” Nisisako

at 24 (emphasis added).

3467. A POSA would also prefer to carry out reactions in microfluidic droplets because its small dimensions allow for reduction of diffusion time for bimolecular reactions. Biomolecular reactions require two molecules to first encounter each other by diffusion or convection-enhanced diffusion. The reaction time and reaction yield for a given reactor are then determined by the diffusion time and then the kinetic time after the molecular encounter. By reducing the diffusion time, a micro-droplet reactor can significantly enhance the reaction yield. *See, e.g.*, Burns (2001) at 10. The reduction of the diffusion time also allows for careful analyses of different kinetic times or kinetic rates, thus allowing for the selection or screening of chemical or biological catalysts. If such reactions involve thermal programming, the low thermal capacitance of droplets also allows very rapid temperature change, thus preventing undesirable by-products.

3468. For example, with protein crystallization, the reduction of diffusion time reduces exposure to non-ideal environments during the random-walk diffusion *See, e.g.*, (Chayen). A large number of small-volume droplets can also enhance selectivity. If the concentration of the droplets is higher than the concentration of interfering agents, the concentration of the interfering agents will be lower in the droplets. *See, e.g.*, Ferrance at 200.

3469. As another example, Lagally explained that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to *increase the speed of these assays* and to *reduce the amount of material and reagents needed*.” Lagally at 565 (emphasis added). Because PCR and other types of reactions rely on reagents that are often in limited supply—for example, sample DNA—the ability to reduce both the amount of material needed for the reaction to occur and the dead volume of the

reaction would have been highly motivating. Ferrance similarly explained that “[t]he same advantages of *reduced time, sample, and reagents* brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions.” Ferrance at 192 (emphasis added). The modification of Corbett, Lagally, Burns (1996), or Wang to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn would decrease operating costs. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

3470. Curcio likewise taught that “[m]inuturization of the fluidic system is beneficial in two ways: it *enhances the speed of thermal equilibration of the reaction mixture*, thus allowing increased flow velocities and faster PCR. Also *analyte volumes are reduced*, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level.” Curcio at 7 (emphasis added).

3471. Vogelstein additionally taught that microfluidic PCR enabled a sample to be diluted into thousands of discrete reaction volumes that each contained either one template PCR molecule or no DNA molecules. Vogelstein at 9236, 9239. A POSA would have found this advantageous because individual-template PCR reactions would have enabled the detection of relatively rare mutations, dislocations, and allelic imbalances. Vogelstein at 9236, 9239.

3472. Reduction in size of the reaction vessel also allows for precise quantification of, for example, nucleic acids and pathogens. As a single template nucleic acid or pathogen can be

placed in a droplet, detection of successful PCR amplification in a given number of droplets allows for digital quantification of, for example, the number of template nucleic acid or pathogens present. For the same reasons, other types of patterns, including *irregular* expression of nucleic acids, could also be quantified. A POSA would have expected that the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform different types of reactions, including PCR, that would enable these applications.

3473. Further, conducting PCR in microfluidic droplets would reduce potential contamination of the reaction, an issue that the prior art had recognized. *See, e.g.*, Corbett at 3:6-12 (“The most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of the assay.”).

3474. It was also well known that decreasing the scale of different types of reactions, including PCR, to microfluidic levels provided the substantial advantage of making reactors portable. For example, Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Kopp at 1047. Further, it was known that portable PCR reactors could aid physicians in the development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Kopp at 1047. Thus, the prior art demonstrated that using the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to perform PCR (and other types of reactions) would have advantageously allowed PCR and other

types of reactions to be performed in point of care diagnostic applications.

3475. Additionally, using the microfluidic reactors for PCR reactions would have substantially increased the tolerance of PCR reactions to primer non-specificity. As of the filing date, it was well known that PCR reactions suffered from the limitation that the primers were not always specific to the sequence of interest but rather could also bind to other sequences. Cha at 526. Because PCR amplification reactions are exponential in nature, PCR would often be ineffective where these other DNA fragments outnumbered the fragments of interest. *Id.* In such circumstances, the amplification products of the former would greatly exceed the amplification of the latter. *Id.* By using multiple droplets, a POSA could reduce the chances of having an uncontaminated DNA template in a single reaction. *Id.* Further, a POSA could conduct exponential amplification of the template without having the intended amplification product compete with unintended amplification products. *Id.*

3476. Moreover, a POSA would have expected the combination of microfluidic droplet reactors and different types of reactions, including PCR, to be successful. For example, in 2001, Lagally et al. provided an overview of the evolution of continuous flow PCR microreactors:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or

continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Lagally at 565-566.

3477. In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct single-molecule DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the

single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

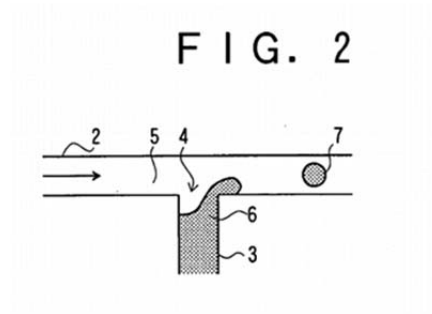
The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Lagally at 566-567. Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. Consistent with the observations in the prior art, a POSA would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR reactions or other autocatalytic reactions.

3478. The fact that several other groups simultaneously developed microfluidic systems that fall within the claims of the Ismagilov patents provides further evidence that a POSA would have both found the combinations described above obvious and would have had a reasonable expectation of success in so combining. For example, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001, more than a year prior to the '091 patent's earliest claimed priority date. *See* Higuchi I-III.

3479. Higuchi I discloses "a process and apparatus for rapidly producing an emulsion and microcapsules in a simple manner." Higuchi I at Abstract. As an example, Higuchi describes

that “[a] process for producing an emulsion includes a step of ejecting a dispersion phase from a dispersion phase-feeding port toward a continuous phase flowing in a microchannel in such a manner that flows of the dispersion phase and the continuous phase cross each other, whereby microdroplets are formed by the shear force of the continuous phase and the size of the microdroplets is controlled.” Higuchi I at [0006]. This is illustrated by Figure 2 in Higuchi I, reproduced below:



Higuchi I at Fig. 2. In the text accompanying the figure, and corresponding with the numbers, Higuchi I describes that “[a] dispersion phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Abstract. The microfluidic droplet system Higuchi and his colleagues developed was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet systems to create droplets from continuously flowing streams of water and oil—and the use of those droplets to conduct reactions—was within the level of skill in the art as of the earliest effective priority date.

3480. As another example, Todd Thorsen (who co-authored the Thorsen reference discussed above) also developed a droplet reactor that falls within the claims of the Ismagilov

patents. Thorsen Thesis at 94-108. The Thorsen Thesis describes the following microfluidic droplet reactor:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

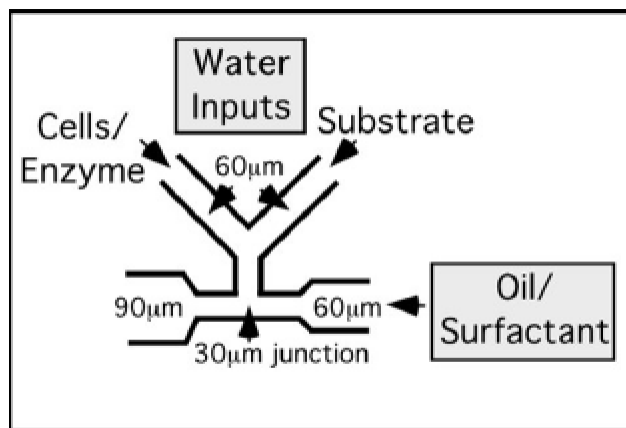


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

3481. The Thorsen thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002, suggesting that Thorsen’s work was performed before this date. The Thorsen Thesis was deposited with CalTech THESIS on December 2, 2002. Thorsen Thesis at 10X-000255686. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov

patents.

3482. A person of ordinary skill in the art would have been further motivated to use two or more aqueous fluids, as described in Kenis, Weigl, Kopf-Sill, Erbacher, and Whitesides to conduct reactions because the art had already described these concepts. For example, Quake explicitly contemplates conducting reactions within droplets using two different aqueous fluids. For example, Quake discloses that:

In another preferred embodiment, the device comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a second droplet extrusion region. A fluid containing a first biological material may pass through the first inlet region so that droplets of the fluid containing the first biological material are sheared into the main channel. A fluid containing a second biological material may pass through the second inlet region so that droplets of the fluid containing the second biological material are sheared into the main channel. In various aspects, the droplets of the first material may mix or combine with the droplets of the second biological material and the first and second biological materials may interact with each other upon mixing. For example, the first biological material may be an enzyme and the second biological material may be a substrate for the enzyme. The interaction of the first and second biological materials may produce a signal that can be detected, *e.g.*, as the droplet passes through a detection region associated with the device.

Quake at ¶ [0018]. Therefore, it was known within the art that using two aqueous fluids to conduct a reaction was useful for separating reagents before droplet formation. *Id.* For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using two or more aqueous fluids to conduct reactions in microfluidic droplets.

3483. A POSA would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Schubert, or Krafft in these microreactor

systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ramsey at 6:49-50 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). For example, Curcio described that perfluorodecalin was utilized as a carrier fluid with small-volume PCR because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity [and thus, high biocompatibility] towards biomolecules.” Curcio at 9. Therefore, a POSA conducting a reaction with a biological molecule in microfluidic droplets, such as PCR, would have used fluorinated oils and fluorinated surfactants with these microfluidic droplet systems. For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

3484. Further, fluorinated oil offers high immiscibility with water and low solubility of biomolecules. *See, e.g.*, Schubert at 97 (“Fluorinated compounds also offer the potential for biomedical applications. For example, . . . fluorinated alkanes are . . . chemically and biologically stable.”); *id.* (“Because fluorocarbons are insoluble in water, however, they are currently administered in the form of emulsions, the formation of which requires the use of surfactants.”). Unlike most mineral oils, fluorinated oil has a density higher than water. Gelest at 19. This higher density allows easy separation of aqueous droplets from the oil when the

emulsion is collected off the substrate.

3485. The art had also already noted that fluorination was preferable for silicon-based microfluidic devices, which have a tendency to swell when exposed to hydrocarbon oils. *See* Quake at [0118] (emphasis added) (“**TEFLON [which contains fluorination] is particularly suitable for silicon elastomer (RTV) channels**, which are hydrophobic and advantageously do not absorb water, but **they may tend to swell when exposed to an oil phase.**”). As Quake noted, “[s]welling may alter channel dimensions and shape, and may even close off channels, or may affect the integrity of the chip, for example, by stressing the seal between the elastomer and a coverslip.” Quake at [0118]. This issue was also prevalent with PDMS, a silicon material that was commonly used to manufacture microfluidic substrates. *See* Quake at [0216] (emphasis added) (“In a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicon rubber or PolyDiMethylSiloxane (PDMS), **preferably PDMS.**”); ’407 patent at 16:59-61 (“Channels may be molded onto optically transparent silicon rubber or polydimethylsiloxane (PDMS), **preferably PDMS.**”).⁵⁶ Unlike other organic oils, fluorinated oil does not cause polymer like PDMS to swell. Holtze at 1632 (“In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.”) (citing Lee). Therefore, a POSA would have been motivated to use fluorinated oils and surfactants to prevent swelling of the polymer substrate.

3486. Importantly, fluorinated oil is far less viscous than other oils, including mineral oils. *See generally* Gelest. Instead, fluorinated oil has a viscosity similar to water. *Id.* Using a fluorinated oil with a microfluidic droplet device would thus allow high-frequency generation of

⁵⁶ I note that this language in the Ismagilov patents was copied almost directly from Quake.

droplets and parallel generation with multiple orifices. The prior art had already shown that high-throughput droplet generation was desirable. *See* Quake at [0079] (“This arrangement can be used to improve throughput or for successive sample enrichment, and can be adapted to provide a very high throughput to the microfluidic devices that exceeds the capacity permitted by conventional flow sorters.”); Quake at [0093] (“Monodisperse droplets may be particularly preferabl[e], e.g., in high throughput devices and other embodiments where it is desirable to generate droplets at high frequency.”). Further, the viscosity of fluorinated oil is insensitive to temperature, which is particular useful for DNA amplification reactions involving temperature changes. This of course includes PCR. Mullis at 9:55-60. For these reasons, a POSA would have been motivated to use fluorinated oil to achieve higher frequency droplet generation. Indeed, fluorinated oil has become the preferred carrier fluid for high-throughput aqueous droplet microfluidics. Autour at Section 4.1.

3487. As the prior art demonstrates, a POSA would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions, including PCR, as taught by Corbett, Lagally, Burns (1996), or Wang.

XIII. INVALIDITY OF THE '083 PATENT

A. Summary of the '083 Patent

3488. The '083 patent is entitled "Device and Method for Pressure-Driven Plug Transport and Reaction." The abstract explains that the invention "provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid." '083 patent at Abstract.

3489. I understand that Bio-Rad is asserting claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent. Of these claims, claims 1, 20, and 31 are independent. Claims 2, 9, 10, 12, and 13 depend on claim 1. Claim 11 depends on claim 10, and claims 21, 22, and 26 depend on claim 20.

3490. The '083 patent issued from Application No. 11/589,700, filed July 26, 2007 (the "'700 application"). The '700 application was a division of the '970 application (which issued as the '091 patent), filed on May 9, 2003. Provisional application No. 60/379,927 was filed May 9, 2002, and provisional application No. 60,394,544 was filed on July 8, 2002.

1. Priority

3491. I understand that Bio-Rad asserts that claims 1-2, 9-10, 12-13, 20, 22, 26, and 31 of the '083 patent were conceived of "no later than February 8, 2002," and relies on RI00106817-18, RI00111308, and RI00111321 to support this assertion. Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 5. I further understand that Bio-Rad asserts that claims 11 and 21 were conceived of "no later than October 30, 2002," relying on RI00111690-1738 to support this assertion. *Id.* I disagree with Bio-Rad's assertions. The cited documents do not demonstrate the inventors had formed their minds the definite and permanent idea of a complete and operative invention as of the dates alleged.

3492. RI00106817-18, RI00111308, and RI00111321, which Bio-Rad relies on to

evidence the conception of claims 1-2, 9-10, 12-13, 20, 22, 26, and 31, appear to be lab notebook entries. These documents do not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as of February 8, 2002. As an initial matter, Bio-Rad has identified no evidence corroborating its apparent assertion that these entries were created on or before February 8, 2002 other than the dates written on these notebook pages (for example, these notes were not witnessed or countersigned by a third party), and one of these entries, RI00111321, bears a date (“3/10/2002”) after February 8, 2002. Further, these documents do not establish that the inventors had possession of every feature recited in claims 1-2, 9-10, 12-13, 20, 22, 26, and 31, or that every limitation of the claim was known to the inventor as of February 8, 2002. For example, all claims in the ’083 patent involve “a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group” (or “a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid”), while the entries at RI00106817-18 contain no references to such carrier fluids. Instead, the entry RI00106817 describes an experiment in which water and dye is pumped through a microchannel with perfluorodecalin, and “water and oil plugs were found to form consistently,” while the entry RI00106818 describes “fluorescence testing” involving flow “over the surface” of a “gold slide coated with a self-assembled monolayer consisting of 5% melamidie and 95% EGS.” Similarly, while all claims in the ’083 patent require a “non-fluorinated microchannel,” and there is no indication in RI00106817-18 or RI00111321 that the inventors were aware of this limitation at the time of those entries, RI00111308 specifically references the use of a fluorinated compound (“PFD”) to “coat the walls” of a microchannel.

3493. As another example, claim 10 recites “the microfluidic system of claim 1, wherein the at least one plug contains at least one reagent for an autocatalytic reaction.” This limitation is

not suggested by RI00106817-18, RI00111308 or RI00111321. Instead, RI00106817 appears to refer to an experiment where streams of water (with dye) were introduced along with perfluorodecalin into a microchannel, with the observed result that “water and oil plugs were found to form consistently.” The reference to an apparently subsequent experiment involving “Fluorescence testing” in RI00106818 is dated February 5, 2002 and contains no reference to reagents for autocatalytic reactions. Nor does RI00111321.

3494. RI00111690–738, which Bio-Rad relies on to evidence conception of claims 11 and 21, appears to be a grant application. This document does not demonstrate that the inventors had formed in their minds a definite and permanent idea of a complete and operative invention as of October 30, 2002, or establish that the inventors had possession of every feature recited in claims 11 and 21 as of that time. For example, both claims require a plug contain[ing] at least one reagent for a “polymerase-chain reaction.” The only discussion of any “polymerase chain reaction” in the cited application, however, is as a general example (along with silver halide photography) of a context in which an autocatalytic reaction may take place. RI00111719.

3495. I understand that Bio-Rad asserts that each of claims 1-2, 9-10, 12-13, 20, 22, 26, and 31 in the '083 patent were reduced to practice no later than January 27, 2003, based on a number of documents. *See* Plaintiffs' Corrected First Supplemental Response to 10X Genomics, Inc.'s Interrogatory No. 1 at 5 (citing documents from Bio-Rad's production). I further understand that Bio-Rad asserts that claims 11 and 21 of the '083 patent were reduced to practice by May 9, 2003, based on the filing of U.S. Patent Application No. 10/434,970 on that date.

3496. While I agree with Bio-Rad's apparent admission that the documents identified by Bio-Rad fail to evidence actual reduction to practice of the alleged inventions claimed in claims 11 and 21 of the '083 patent, I also believe that these documents fail to evidence actual reduction

to practice of the other asserted claims of the '083 patent. Entirely to the contrary, these documents describe particular limitations of these claims in entirely aspirational terms.

3497. Claims 1, 20, and 31 of the '083 patent, for example, recite that “fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.” While a grant application dated January 27, 2003 cited by Bio-Rad states that “[t]he value of the surface tension of the oil/water interface has to be sufficiently high in order to maintain a low value of Ca ,” by contrast, it further notes that “[f]luorosurfactant/water interfaces for water-insoluble fluorosurfactants *have not been characterized*,” that “we *estimate* that these surfactants will provide reduction of surface tension similar to that of Span on hexane/water interface (-20 mN/m),” and states a future intention to “measure surface tensions of the aqueous/fluorous interfaces in the presence of fluorosurfactants using the hanging drop method.” RI1117773 (emphasis added).

3498. Similarly, for example, while claim 9 of the '083 patent recites “[t]he microfluidic system of claim 1, wherein the fluorinated surfactant comprises an oligoethylene glycol,” the same grant application describes “*propos[ing] to use*,” not *having* used, “oil-soluble fluorosurfactants terminated with oligoethylene groups to create interfaces in our microfluidic devices.” RI00111772 at 34 (emphasis added).

3499. I understand that Bio-Rad has provided no evidence of the inventors’ diligence in reducing the alleged inventions of the '083 patent to practice after Bio-Rad’s alleged dates of conception, and consequently that there is no evidence that any claim of the '083 patent would be entitled to priority as of Bio-Rad’s alleged dates of conception, even if these dates were uncontested (which they are not). Plaintiffs’ Corrected First Supplemental Response to 10X

Genomics, Inc.’s Interrogatory No. 1 at 5. While Bio-Rad cites a number of documents “*see also*” and “*see, e.g.,*” many of these documents do not appear to relate to diligence, and none establish diligence. Plaintiffs’ Corrected First Supplemental Response to 10X Genomics, Inc.’s Interrogatory No. 1 at 5. For example, RI00111541-57, RI00111558-70, and RI00111572-79 are undated. RI00111688-89 appears to be an email chain between George Whitesides and Rustem Ismagilov dated July 21-22, 2002. This email refers to “thinking” about potential surfactants. It does not describe work which had been or was currently being performed. RI00111739-793 is dated on or after the alleged date of reduction to practice for many of the asserted claims. RI00111677-78 appears to be an email from Heinrich Jaeger and Rustem Ismagilov dated April 24, 2002. The email speaks, in the future tense of potential future projects that “use microfluidics.” It does not describe work which had been or was currently being performed. RI00111684-87 appears to be an email chain between Rustem Ismagilov, Vince Turitto, and Connie Hall dated March 30, 2002 to April 24, 2002. This email chain again speaks of potential future work, not work that had been or was currently being performed. RI00111571 appears to be typed notes titled “Microfluidics Assessment 3/15/2002” that refers generally to “[f]orming plugs.” RI00111794-97 appears to be a draft article or abstract dated September 25, 2002. RI00111580-636 appears to be a copy of the Ismagilov ’927 provisional application filed on May 9, 2002. RI00111679-83 appears to be an email chain between James Norris Jr. and Rustem Ismagilov dated between October 3-19, 2002. RI00111673-76 appears to be an email chain between Rustem Ismagilov and Donna Blackmond dated October 7-15, 2002. While these documents appear to describe work that had been performed relating to “plugs” it is not clear when this work was performed. RI0011571 is dated a month after the alleged conception date for many of the asserted claims. RI00111794-97 is dated over seven months after the alleged

conception date for many of the asserted claims. RI00111580-636 is dated over three months after the alleged conception date for many of the asserted claims. Further, as discussed in Section VII.A, much of this application was copied from a PCT application related to Quake, and accordingly does not evidence Ismagilov's diligence. RI00111679-83 is dated over eight months after the alleged conception date for many of the asserted claims. RI00111673-76 is dated over eight months after the alleged conception date for many of the asserted claims. Further, as set forth in **Exhibit 2**, I have reviewed various lab notebooks from Dr. Ismagilov's lab dated from May 9, 2003. None of these notebooks suggest that any work was done to reduce the inventions claimed in claims 11 and 21 of the '083 patent to practice in the nearly seven months between the alleged date of conception (October 30, 2002) and the alleged date of reduction to practice (May 9, 2003). For example, none of these notebooks include experiments or work relating to conducting PCR in plugs.

3500. Should Bio-Rad be permitted to present additional evidence or contentions regarding conception, diligence, or reduction to practice (and I understand that 10X's position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

B. Invalidity Overview

3501. As shown in further detail below, my opinions regarding the '083 patent include the following:

- All asserted claims are invalid under Section 112 for lack of proper written description, lack of enablement, and/or indefiniteness.
- All asserted claims are obvious in light of Quake under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Shaw Stewart under Section 103 (either alone or in combination with other references).

- All asserted claims are obvious in light of Burns (2001) under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Nisisako under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Thorsen under Section 103 (either alone or in combination with other references).
- All asserted claims are obvious in light of Seki under Section 103 (either alone or in combination with other references).

C. Invalidity Under 35 U.S.C. § 112

3502. As described in further detail below, it is my opinion that the asserted claims of the '083 patent are invalid under 35 U.S.C. § 112.

1. Written Description

3503. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that certain claims of the '083 patent are invalid for lack of written description.

3504. Claim 10, for example, requires a **"reagent for an autocatalytic reaction."** Claim 11, for example, requires **"a reagent for an autocatalytic reaction" "wherein the autocatalytic reaction is polymerase-chain reaction."** Claims 20-22 and 26 of the '083 patent, for example, require a **"reaction."** I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Bio-Rad appears to be taking the position that "reaction" is far broader than what was disclosed in the '091 patent. Based on Bio-Rad's 4(c) disclosures, Bio-Rad contends that 10X performs a "DNA amplification reaction" in its 10X GemCode™ platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,889,083 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 67, 75, 109,

128; *see also* Appendix E to Plaintiffs’ Supplemental Response to Interrogatory No. 4 at 69, 87, and 148. I have reviewed the ’083 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. The specification includes a single reference to a DNA amplification reaction: “Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.” ’083 patent at 44:36-39. There is no mention in the ’083 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. Indeed, there is no indication that the inventors of the ’083 patent contemplated any DNA amplification reaction beyond the basic (and well-known) PCR reaction. Nor has Bio-Rad identified any disclosure in the ’083 patent specification that discloses other DNA amplification reactions. This infirmity affects claims 10, 11, 20-22, and 26.

3505. There is also, for example, no adequate description of performing a “**reaction**” in plugs *outside of a substrate*, including, for example, a DNA amplification reaction outside of a substrate. I have reviewed the ’083 patent, and it does not contain any disclosure that would justify the scope Bio-Rad has accused. There is no indication that the inventors of the ’083 patent contemplated performing a DNA amplification reaction in plugs outside of the substrate. Nor has Bio-Rad identified any disclosure in the ’083 patent specification that discloses a DNA amplification reaction in plugs outside of the substrate. This infirmity affects claims 10, 11, 20-22, and 26.

3506. Bio-Rad has taken the following position:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can

be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X’s Interrogatory No. 3. As an initial matter, none of the identified reactions are DNA amplification reactions. Further, as discussed below, Bio-Rad has not identified any teaching in the ’083 patent that would convey to a POSA that the inventors had possession of a surfactant that would stabilize droplets and prevent droplet coalescence to allow for a “reaction” in plugs outside of the substrate, let alone a DNA amplification reaction outside of the substrate.

3507. None of the “embodiments in which droplets are captured in a capillary tube” (the “capillary tube embodiments”) identified by Bio-Rad, are included in the specification of the ’083 patent.

3508. In addition to the “capillary tube embodiments,” Bio-Rad cites to a portion of the specification as “*contemplat[ing]* collection of droplets and removal from the chip,” First Supplemental Response to 10X’s Interrogatory No. 3 (emphasis added):

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

’083 patent at 16:63-17:3 (emphasis added). As an initial matter, this section “contemplates” collecting “*solution*” not *plugs* or *droplets*. Further, the specification provides no working

examples describing the collection of droplets in “a standard 1.5 ml centrifuge tube” or “[c]ollection . . . using micropipettes”⁵⁷ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalescence to allow such collection, and subsequent DNA amplification outside of the substrate.

3509. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. However, as discussed below, the surfactant depicted in Figure 24 would not stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in droplets off in plugs outside of the substrate.

3510. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927,⁵⁸ these applications lack adequate description of performing a “**reaction**” including, for example, a DNA amplification reaction. have reviewed the ’544 and ’927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. The specifications of the ’544 and ’927 provisional applications do not include a single reference to a DNA amplification reaction. There is no mention in the ’544 or ’927 provisional applications of (for example) *any* DNA amplification reactions, let alone the details necessary to carry out said reactions. Nor has Bio-Rad identified any disclosure in the ’544 or ’927 specifications that

⁵⁷ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 (“Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.”); *see also* Quake at [0148].

⁵⁸ I understand that Bio-Rad is not currently claiming priority to these applications. Plaintiffs’ Corrected Response to Interrogatory No. 1 at 5.

discloses other DNA amplification reactions. This infirmity affects claims 10, 11, 20-22, and 26.

3511. The applications also lacks adequate description of a “**reaction**” in plugs outside of a substrate, including, for example, a DNA amplification in plugs outside of a substrate. I have reviewed the ’544 and ’927 provisional applications, and they do not contain any disclosure that would justify the scope Bio-Rad has accused. Nor has Bio-Rad identified any disclosure in the ’544 and ’927 provisional applications that discloses a DNA amplification reaction in plugs outside of the substrate. This infirmity affects claims 10, 11, 20-22, and 26.

3512. Bio-Rad has taken the following position in its Response to 10X’s Interrogatory No. 3:

The patents-in-suit expressly contemplate embodiments where reactions take place *off* the chip. Specifically, that patents-in-suit describe embodiments in which droplets are captured in a capillary tube, which is a tube that can be “up to several millimeters” in diameter. . . . In such embodiments, the capillary tube can be removed from the microfluidic chip (which is constructed from material referred to as “PDMS”), sealed in wax, and transferred to an incubator for a chemical reaction.

Numerous examples in the specification utilize this off-chip approach. . . . [and] all patents-in-suit include disclosure of collecting droplets using centrifuges or micropipettes

First Supplemental Response to 10X’s Interrogatory No. 3.

3513. But the ’544 and ’927 provisional applications do not describe a single “embodiment in which droplets are captured in a capillary tube,” let alone a DNA amplification reaction in plugs outside of the substrate.

3514. The specifications of the ’544 and ’927 provisional applications state:

Thus, devices according to the invention may have a plurality of analysis units that can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

'544 application at 28:22-26; '927 provisional application, 27:14-23. Again, this section speaks to collecting "*solution*" not *plugs* or *droplets*. Further, the specification provides no working examples describing the collection of droplets in "a standard 1.5 ml centrifuge tube" or the "[c]ollection . . . using micropipettes"⁵⁹ and the surfactants described in the specification would not stabilize droplets or prevent droplet coalesce to allow such collection.

3515. Bio-Rad states that "the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip" and cites to Figure 24. First Supplemental Response to 10X's Interrogatory No. 3. The '544 and '927 provisional applications do not include this figure, or any related discussion. The '544 and '927 provisional applications note that "exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water," '927 provisional application at 12:16-17; '544 application at 12:19-13:5,⁶⁰ and describe the following "[p]referred surfactants":

⁵⁹ In fact, this language appears to have been copied from Quake PCT. Quake PCT at 44:16-20 ("Thus, devices of the invention having a plurality of analysis units can collect the solution from associate branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adopted for receiving, for example, a segment of tubing or sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes."); *see also* Quake at [0148].

⁶⁰ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 ("The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water."); *see also* Quake at [0117].

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

’544 application at 12:19-13:3; ’927 provisional application at 10:31-11:15.⁶¹ However, as

⁶¹ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

discussed below, none of the surfactants described would stabilize droplets or prevent droplet coalescence such that a DNA amplification reaction could be performed in plugs outside of the substrate.

2. *Enablement*

3516. As described in further detail above, I have reviewed various documents regarding Bio-Rad's infringement position in this case. Based on these documents, it is my opinion that certain claims of the '083 patent are invalid for lack of enablement.

3517. Claim 10, for example, requires a **"reagent for an autocatalytic reaction."** Claim 11, for example, requires **"a reagent for an autocatalytic reaction" "wherein the autocatalytic reaction is polymerase-chain reaction."** Claims 20-22 and 26 of the '083 patent, for example, require a **"reaction."** I understand the Court has construed "reaction" as: "Physical, chemical, biochemical or biological transformation. The location of reactions is not limited to the substrate." Claim Construction Order at 1. Based on Plaintiffs' 4(c) disclosures, Bio-Rad contends that 10X performs a "DNA amplification reaction" in its 10X GemCode™ platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,889,083 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 67, 75, 109, 128; *see also* Appendix E to Plaintiffs' Supplemental Response to Interrogatory No. 4 at 69, 87, and 148. But the specification of the '083 patent does not enable the full scope of the limitation, at least under Bio-Rad's actual and/or apparent application of the claims, without undue experimentation. The claims purport to cover *all* DNA amplification reactions in plugs (whether known or unknown at the time of Ismagilov's alleged invention), but the specification includes a single reference to a DNA amplification reaction: "Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used

in the biological sciences.” ’083 patent at 44:36-39. The specification does not include a working example of a PCR reaction in plugs. And there is no mention in the ’083 patent of (for example) other DNA amplification reactions, let alone the details necessary to carry out said reactions. The ’083 patent fails to disclose, teach, or suggest how to conduct every “DNA amplification reaction,” and particularly, the “DNA amplification reactions” allegedly performed by 10X,⁶² within plugs.

I understand that these techniques were developed by 10X years after the priority date of the Ismagilov patents.

3518. Further, the claims purport to cover *all* DNA amplifications in plugs (whether known or unknown at the time of Ismagilov’s alleged inventions), including DNA amplification reactions in plugs *outside of the substrate*. But the specification of the ’083 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. The specification does not enable DNA amplification reactions in plugs *outside of the substrate*. The specification does not include a single working example of a DNA amplification reaction, let alone a DNA amplification reaction outside of the substrate. Surfactants that would enable a POSA to conduct biological reactions within microfluidic droplets outside of the substrate, let alone DNA amplification reactions outside of the substrate, are not described in specification of the ’083 patent and were not even available as of the alleged priority date of the ’083 patent. In fact, surfactants appropriate for this use were not developed or described until 2008—seven years after Ismagilov’s alleged invention.

3519. As discussed above, Bio-Rad contends that 10X performs a “DNA amplification

⁶² I have not been asked to provide, and have not formed an opinion on whether or not the reactions performed in 10X’s products are “DNA amplification reactions.”

reaction” in its 10X GemCode™ platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.”

3520. The surfactants disclosed in the Ismagilov patents would not stabilize droplets under these conditions.

3521. In order to conduct biological assays within microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, preventing coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Holtze at

1632.⁶³ “These requirements [were] particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluortelomer-tails (typically perfluorinated C₆ to C₁₀) . . . do not provide sufficient long-term emulsion stability.” *Id.*⁶⁴ Even as of 2008, years after the priority date of the ’083 patent, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.*

3522. Bio-Rad has taken the position that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. But none of the surfactants disclosed in the specification of the ’083 patent, including the surfactants disclosed in Figure 24, meet the requirements set forth above.

3523. As set forth in the specification, “FIG.24 shows a reaction scheme that depicts examples of fluorinated surfactants that form monolayers that are: (a) resistant to protein adsorption; (b) positively charged; and (c) negatively charged. Fig. 24b shows a chemical structure of neutral surfactants charged by interactions with water by protonation of an amine or guanidinium group. FIG 24c shows a chemical structure of neutral surfactants charged by interactions with water deprotonation of a carboxylic acid group.” ’083 patent at 5:22-30.

⁶³ Holtze was authored by individuals from Harvard University, Universit`a del Salento, Lecce, Italy, and Raindance Technologies, Inc. Holtze at 1632.

⁶⁴ When conducting biological assays in droplets, “it is attractive to use a fluorocarbon oil as the continuous phase” and accordingly, a fluorosurfactant to “ensur[e] that drops are stable.” Holtze at 1632.

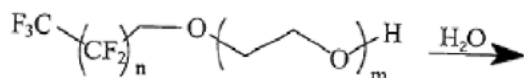


FIG. 24A

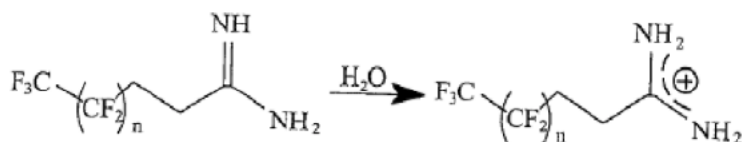


FIG. 24B

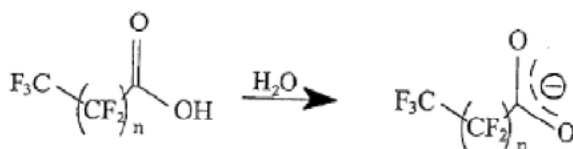


FIG. 24C

Fig. 24

3524. Specifically, Figure 24a “depicts a “fluorinated surfactants containing perfluoroalkyl chains [(red)] and an oligoethylene glycol head group [(blue)].” ’083 patent, 8-10.

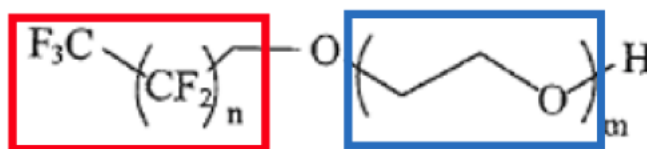


FIG. 24A

3525. The surfactant depicted in Figure 24a is commercially available under the trade name Zonyl.” See ’083 patent at 20:29-31(“Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)”); ’407 patent at 76:64-66 (“A fluorinated carrier fluid was a saturated solution of FSN surfactant in FC3283.”).

3526. Figure 18, depicts the same fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group. ’083 patent at 56:56-58 (“In FIG. 18, plugs are formed in the presence of several solutions of surfactants that possess different functional groups

(left side of the diagram))” (annotation added).

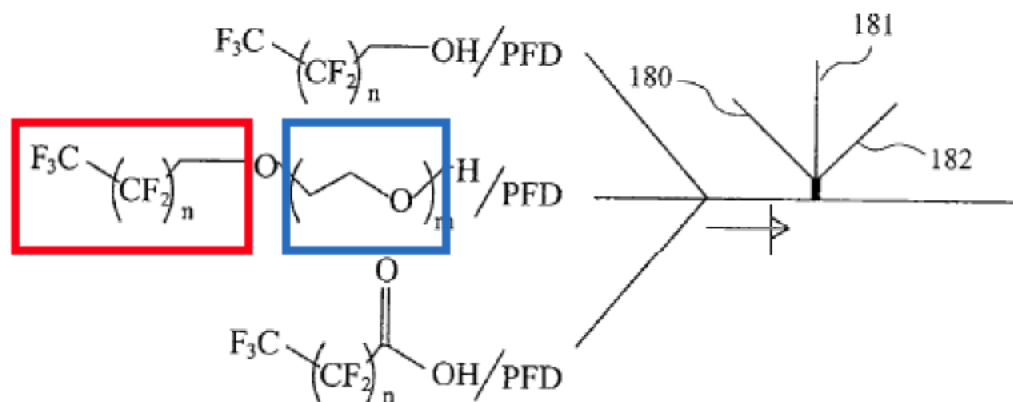


Fig. 18

3527. Unlike the ionic surfactants depicted in Figure 24b and 24c, the surfactant depicted in Figure 24a meets the second requirement set forth above. It will “produce a biologically inert interior surface for the water drops.” As described in the specification of the ’083 patent: “[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces.” ’083 patent at 35:41-44. Further, this OEG head group is non-ionic as required for biological assays. Holtze at 1632. But this surfactant does not meet the first requirement set forth above for performing biological assays in droplets. Specifically, it would not “provide stability to the drops, preventing coalescence.”

3528. The surfactant depicted in Figure 24a contains “a “perfluoroalkyl chain[] and an oligoethylene glycol head group.” ’083 patent at 72:9-10. A perfluoroalkyl chain (also referred to as a “perfluoroalkyl tail” of “fluorotelomer-tail”) is not sufficient to stabilize droplets outside of the substrate. As described by Holtze *et al.* “[s]urfactants with short fluorotelomer-tails” like the perfluoroalkyl chain depicted in Figure 24a, “do not provide sufficient long-term emulsion

stability.” Holtze at 1632.

3529. I understand that Dr. Jeremy Agresti, Bio-Rad’s R&D Director and a co-author on Holtze et al., confirmed this point. Dr. Agresti was questioned regarding the text copied below from Holtze et al.:

However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C₆ to C₁₀) have been used, but do not provide sufficient long-term emulsion stability. Fluorosurfactants with longer fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632 (internal citations omitted).

3530. Dr. Agresti confirmed that these statements were accurate at the time they were written in 2008. Agresti Tr. 199:9-13 (“Q. Do you believe that the statements that are made in the article that you just read, those portions that the article that you just read, do you believe

those are accurate? A. Yeah, at the time for sure.”).

3531. Further in reference to the following statement in Holtze et al.: “Biological assays thus demand fluorosurfactants with non-ionic head groups; however, there are currently no such surfactants available,” Dr. Agresti confirmed that as of 2008 there were no “flourosurfactants with nonionic head groups that would stabilize and emulsion long term.” Agresti Tr. 202:2-13 (“Q. And it was true that as of – as of the date of this article, which was 2008, that at least to your knowledge that there were no nonionic fluorosurfactants with nonionic head groups? A. That could stabilize an emulsion long term. We knew that there were fluoro surfactants with nonionic head groups. Q. [W]hat was not known was that there were fluoro surfactants with nonionic head groups that would stabilize an emulsion long term. A. Yes, that’s right.”). Dr. Agresti further confirmed that a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group, specifically Zonyl, “doesn’t stabilize droplets for PCR.” Agresti Tr. 203:10-19 (Q. Are you familiar with a surfactant known as . . . ZONYL? A. Yes. Q. Has Bio-Rad used that surfactant? A. I can’t say. It’s not in any product. As far as I know it’s never been in any product. Q. Why not? A. As far as I know it doesn’t stabilize droplets for PCR.”).

3532. I understand that named inventor of the ’083 patent Mr. Lewis Spencer Roach, who testified that his “primary contribution” was to developing “fluorinated surfactant[s] with hydrophilic head group” Roach Tr. 26:21-23, also confirmed the point that a surfactant with a perfluoroalkyl chain and an oligoethylene glycol head group, like Zonyl would, not stabilize droplets long term. Mr. Roach testified that “other groups have done a lot of work on preventing coalescence using surfactants” but “I did not personally perform that research.” Roach Tr. 78:15-20. When asked whether “other groups” mean “other people in Dr. Ismagilov’s lab,” Mr. Roach answered that he “believe[d] it was outside of Ismagilov’s group” Roach Tr. 78:21-79:2. Mr.

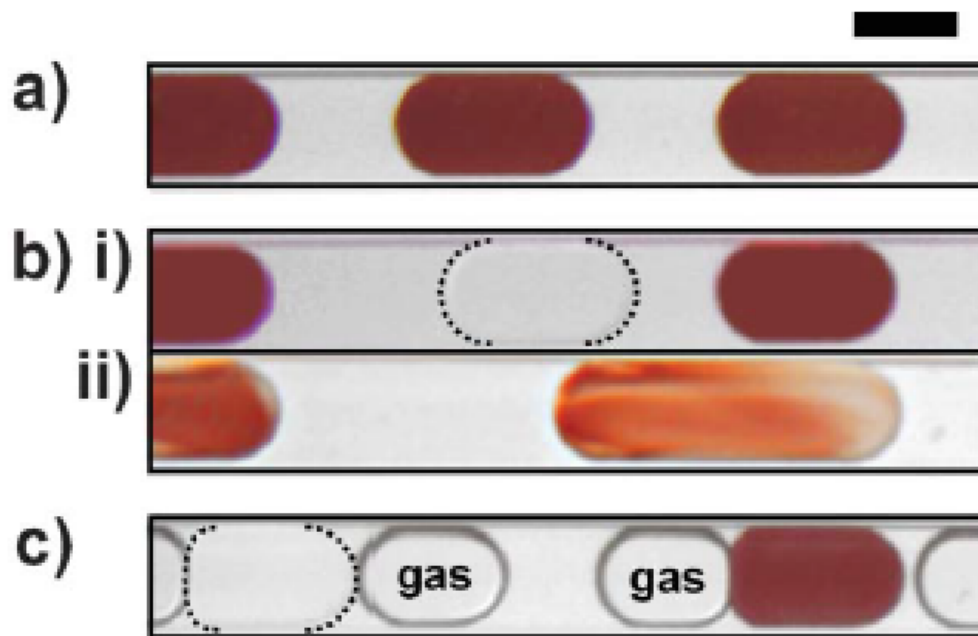
Roach further testified that the “Rf-OEG surfactant is not optimized for preventing coalescence . . . [t]here are other hydrophilic head groups that are better at controlling adsorption than a simple oligo (ethylene glycol) head group. I think other people have made these.” Roach Tr. at 79:3-12.⁶⁵ Mr. Roach later confirmed that the “other people” he was referring to were Holtze et al. in 2008. Roach Tr. 80:4-11 (“A. I believe [Exhibit 129 (Holtze et al.)] is what I was just referring to, that other groups had optimized surfactants to – give me just a second. I want to read the conclusions in this paper here. Q. Certainly. A. Yes. This is where I was discussing other groups that have optimized surfactants to prevent coalescence or merging of plugs.”).

3533.

⁶⁵ The “Rf-OEG” surfactant is a surfactant with a perfluoroalkyl chains and an oligoethylene glycol head group. *See* Roach Depo. Ex. 127 (“Perfluorinated-tail, oligoethylene glycol derivatized molecules (Rf-OEG) were selected as a neutral and hypothetically biocompatible surfactants.”); Roach Tr. 51:22-23 (“A. I synthesized the surfactant described in [Exhibit 127], particularly the Rf-OEG surfactant.”).

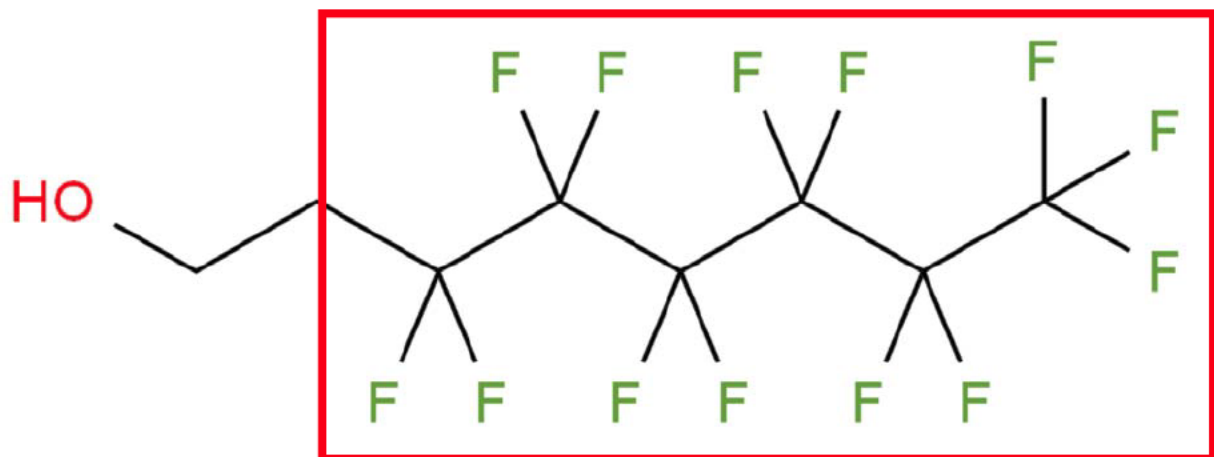
3534. I agree with Agresti's, Roach's, and Hindson's statements above regarding fluorinated surfactants containing perfluoroalkyl chains and an oligoethylene glycol head group, like Zonyl. Such surfactants would not provide stability to drops and prevent coalescence to allow for DNA amplification reactions in microfluidic droplet outside of the substrate.

3535. Dr. Ismagilov himself recognized the potential for coalescence, even between plugs within the substrate. As explained by Dr. Ismagilov, "[d]uring flow, plugs with different chemical composition may move relative to the carrier fluid at different rates and thus move relative to one another allowing adjacent plugs to coalesce (Fig. 2(b))." Adamson at 1181.



3536. Figure 2b above depicts plug coalescence between "[p]lugs of distinct chemical composition." Adamson at 1181. The carrier fluid is "FC-3283 10:1 PFO (v/v) throughout."

Adamson at 1181. FC-3283 is a fluorinated oil. PFO or 1H,1H,2H,2H-perfluorooctanol is a fluorinated surfactant. The chemical formulation of PFO is depicted below:

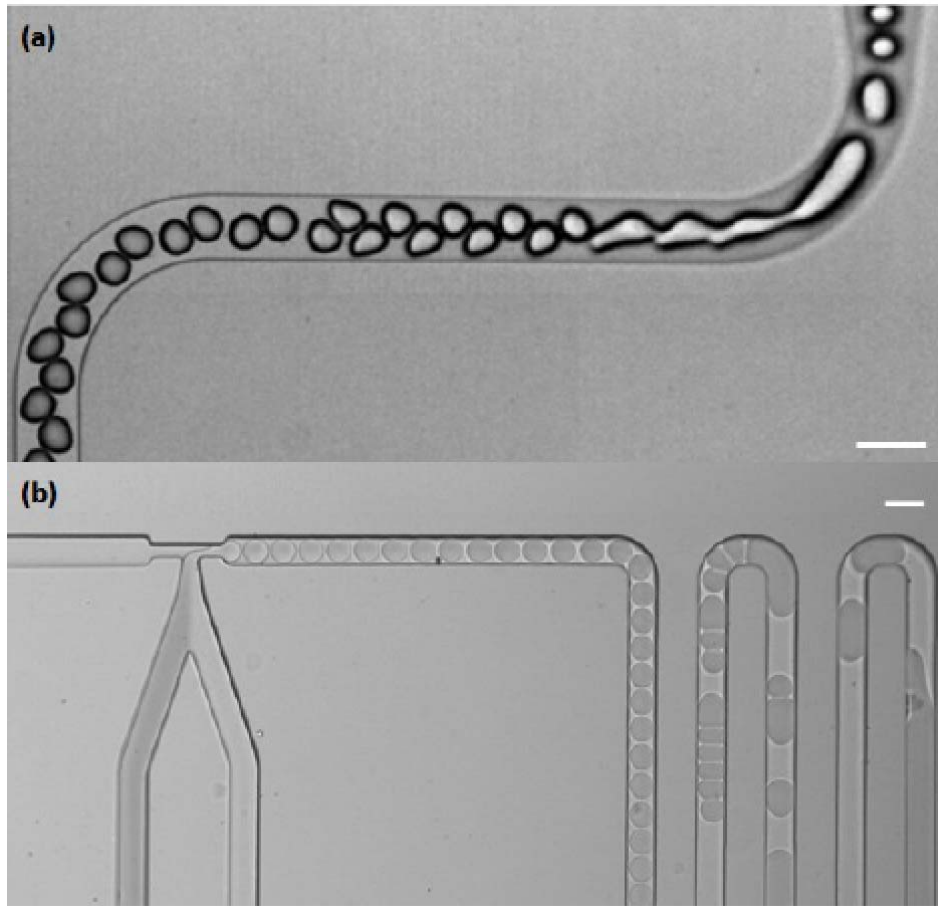


3537. Like Zonyl, PFO contains a short perfluoroalkyl tail (red).

3538. As explained by Dr. Ismagilov, this surfactant was insufficient to prevent coalescence even within the substrate. *See* Adamson at 1181. Instead “[t]o prevent coalescence, gas bubbles [were] introduced as spacers between plugs to (1) minimize the relative motion of plugs and (2) to act as a physical barrier to prevent the coalescence of adjacent plugs during flow and splitting.” Adamson at 1181.

3539. This potential for droplet coalescence was later described as “[u]ncontrolled.” “Cho Thesis “at Fig. 3.1. This thesis explained: “Perfluorodecalin and 1H,1H,2H,2H-perfluoro-1-octanol combination was used for studying protein crystallization by Ismagilov and his coworkers. The perfluorinated oil and surfactants are advantageous for microdroplet based biochemical applications as they are lipophobic, inert, insoluble in water and compatible with many biochemical molecules. Unfortunately, droplets in perfluorodecalin oil with 1H,1H,2H,2H-perfluoro-1-octanol were not stable and merged with each other under pressure (Figure 3.1(b)[D]).” Cho Thesis at 51. The “[d]roplet generation and uncontrolled coalescence of droplets

in perfluorodecalin with 5% v/v 1H, 1H, 2H, 2H-perfluoro-1-octanol” observed is depicted in (b) below:



3540. “Fluorosurfactants with longer fluorocarbon tails” are required for “long-term stabilization” to perform biological assays. Holtze at 1632. As further described by Holtze et al., as of 2008, no such surfactant existed in 2008:

However, the only available PFPE-based surfactants have ionic headgroups, e.g. poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity. Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Holtze at 1632. Holtze et al. disclose examples of fluorinated surfactants meeting the two requirements set forth above. These surfactants comprise non-ionic polyethylene glycol head groups and perfluorinated polyether tails. Holtze at 1; Figure 2.

3541.

I understand that RainDance's droplet products utilize a "biocompatible surfactant, PEG-PFPE block copolymer." Plaintiffs' First and Second Supplemental Response to Interrogatory No. 5. Bio-Rad's droplet products utilize "Krytox K225 (0.58mM) + perfluorodecanol (0.625 mM) or the BRDG3 triblock fluorosurfactant." Plaintiffs' Third and Fourth Supplemental Response to Interrogatory No. 5.

3543. Further, to the extent that Bio-Rad claims priority to U.S. Provisional Application 60/394,544 or U.S. Provisional Application No. 60/379,927, the specifications of the '544 and '927 provisional applications do not enable a person of skill in the art to conduct biological reactions within microfluidic droplets outside of a microfluidic substrate without undue experimentation. Surfactants necessary to conduct biological reactions within microfluidic

droplets outside of a microfluidic substrate are not described in specification of the '544 or '927 provisional applications and were not even available as of the filing date of the '544 or '927 applications.

3544. Bio-Rad states that “the specification of the patents-in-suit includes detailed teaching regarding surfactants that can be used to stabilize droplets as they are removed from the chip” and cites to Figure 24. First Supplemental Response to 10X’s Interrogatory No. 3. The '544 and '927 provisional applications do not include this figure, or any related discussion. The '544 and '927 provisional applications note that “exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water,” '927 provisional application at 12:16-17; '544 application at 12:19-13:5,⁶⁶ and describe the following “[p]referred surfactants”:

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants

⁶⁶ Again, this language appears to have been copied from Quake PCT. Quake PCT at 35:18-20 (“The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.”); *see also* Quake at [0117].

are generally less preferably [sic] for certain embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

'544 application at 12:19-13:3; '927 provisional application at 10:31-11:15.⁶⁷ In the context of conducting biological assays in microfluidic droplets outside of the substrate, each of the surfactants listed—excluding “fluorinated oil” discussed separately below—would be considered an aqueous soluble surfactant by a POSA, meaning they are introduced in the aqueous phase instead of the oil phase. To conduct biological assays in microfluidic droplets outside of a substrate, a POSA would understand that a continuous phase comprised of a fluorinated oil is preferred, if not necessary. Holtze at 1632. The listed surfactants are non-fluorinated and as such are not soluble in fluorinated oil. Therefore, to use one of the listed surfactants in a system comprising a fluorinated oil, the surfactant needs to be introduced into the aqueous phase. However, when present in the aqueous phase these surfactants would be disruptive to emulsion stability. The hydrophobic portions of these surfactant molecules cause them to populate the

⁶⁷ This language also appears to have been copied from Quake PCT. Quake PCT at 28:7-23 (“Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span 80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl, and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerol esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, *etc.*) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactant such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably [sic] for many embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.”); *see also* Quake at [0095]

aqueous-fluorinated oil droplet boundary, displacing any stabilizing fluorinated surfactant molecules present. This process leads to droplet coalescence rather than stabilization.

3545. “Fluorinated oil” while soluble in fluorinated oil, also would not stabilize droplets to conduct biological assays in microfluidic droplets outside of the substrate. Holtze at 1632 (“[I]t is attractive to use a fluorocarbon oil as the continuous phase However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable.”).

3. *Indefiniteness*

3546. It is my opinion that the claims of the ’083 patent are invalid as indefinite because the ’083 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention.

3547. For example, claims 2 and 26 of the ’083 patent, purport to cover a microfluidic system wherein “**at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.**” But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “at least one of a cell, a virion, an enzyme, DNA, and RNA.” For example, does the claim require that “at least one of” *each* of the listed categories, including a cell, a virion, an enzyme, DNA, and RNA is contained in at least one plug, or whether the claim requires “at least one of” a cell, a virion, an enzyme, DNA or RNA in at least one plug.

3548. As another example, the claims of the ’083 patent purport to cover a microfluidic system comprising at least one plug wherein “**the surface tension at the plug-fluid/microchannel wall interface** is higher than [the] surface tension at the plug-fluid/carrier fluid interface.” But the specification and prosecution history fail to inform, with reasonable certainty what constitutes “the plug-fluid/microchannel wall interface.” In a working system, no such “interface” exists. More specifically, a POSA would understand that in a working droplet

system a three-phase contact line does not exist. Such three-phase contact line would be necessary to determine “the surface tension at the plug-fluid/microchannel wall interface.”

3549. For example, Adam Lowe, a Staff Scientist at 10X, was questioned about the following image:

D. Invalidity Based on Prior Art

1. Obviousness

(a) Invalidity Based on Quake

3551. It is my opinion that Quake discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

3552. Claim 1 recites: “**A microfluidic system.**”

3553. Quake satisfies this limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

3554. Claim 1 further recites: “**a non-fluorinated microchannel.**”

3555. Quake satisfies this limitation. For example, Quake states that “[c]hannels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, or from silicon-urethane composites such as those available from Polymer Technology Group (Berkeley, Calif.), e.g., PurSil™ and CarboSil™.” Quake at [0118]. Quake also discloses that “[i]n a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS.” Quake at [0216].

3556. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a**

fluorinated surfactant comprising a hydrophilic head group in the microchannel.”

3557. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3558. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (emphasis added) (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (*preferably a non-polar fluid such as decane or other oil*)

in the main channel.”).

3559. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

3560. Quake also discloses fluorinated oils and fluorinated surfactants. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

3561. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

3562. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Quake. West et al., Surfactants and Subsurface Remediation, *Environ. Sci. Technol.*, 26(12): 2324-2330 (1992) (“West”) (10X-000002406-2412) at 2324. Therefore, a POSA would have understood that Quake’s disclosure of fluorinated surfactants included fluorinated

surfactants with hydrophilic headgroups.

3563. While it is my opinion that Quake discloses a fluorinated oil and fluorinated surfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3564. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3565. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is

required.” *Id.* at 6:46-50.

3566. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3567. Claim 1 further recites: “**at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.**”

3568. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003]; *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3569. Quake also discloses that the droplets are substantially encased by the carrier fluid. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each*

other by oil.” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

3570. Claim 1 further recites: “**wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

3571. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added). Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion.

3572. In 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng, S., and Williams, R. “Controlled Production of Emulsions Using a Crossflow Membrane”, Trans I Chem E, 76: 894-901 (1998) (“Peng”) (10X-000255440-47) at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension . . . If the contact angle . . . is too

small or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . . “). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3573. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid.

3574. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous. Quake described that “silicon elastomer (RTV) channels, which are hydrophobic and advantageously do not absorb water, . . . may tend to swell when exposed to an oil phase . . . Urethane substrates do not tend to swell in oil but are hydrophilic, they may undesirably absorb water, and tend to use higher operating pressures [to ensure the oil flow rate sustains an encapsulating film of carrier oil].” Quake at [0118]. A POSA would have understood that a hydrophilic urethane surface could enable droplet contact with the wall, if the oil flow rate was not high enough. Quake further states that “the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol/vol) of either 0.5, 1.0 or 2.0%. The devices were equilibrated prior to crossflow by priming the out flow channel with oil to

eliminate interactions of the aqueous [plug] phase with the hydrophilic urethane walls of the channel.” Quake at [0300]. Therefore, Quake teaches this limitation.

3575. While it is my opinion that Quake discloses a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3576. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3577. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

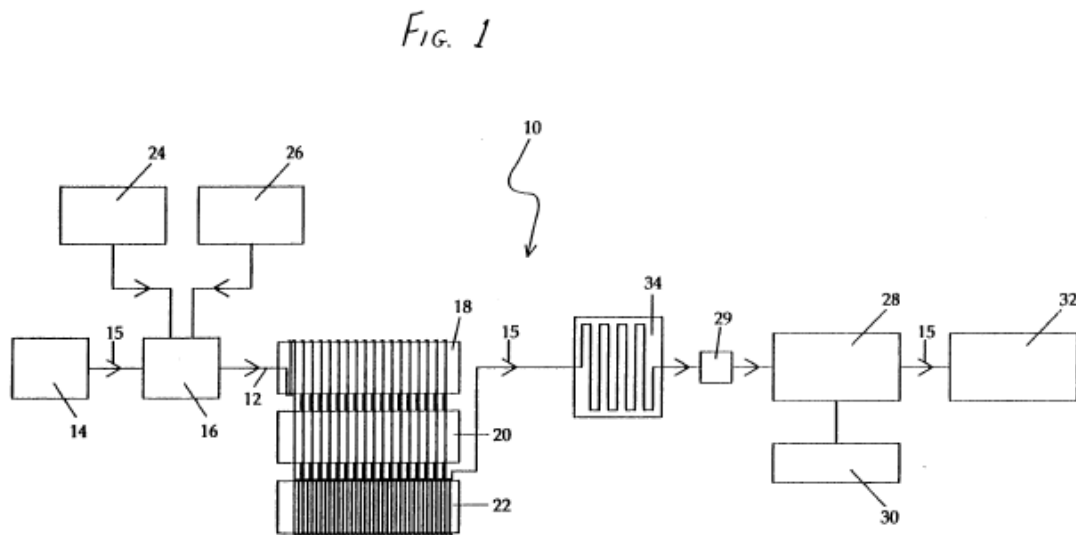
3578. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3579. Claim 2 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.**”

3580. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles*, or mixtures thereof.” Quake at [0021] (emphasis added). Quake defines “polynucleotide” as including “*double and single stranded RNA and DNA*.” Quake at [0052] (emphasis added).

3581. While it is my opinion that Quake discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application,

for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

3582. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally,

“[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3583. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3584. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of

Success), and all references cited therein.

(iii) *Claim 9*

3585. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3586. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

3587. Quake satisfies this element. For example, Quake discloses that “non-limiting examples of nonionic surfactants which may be used include . . . long chain carboxylic acid esters (for example, . . . *polyoxyethyle glycol esters*, etc.).” Quake at [0095].

3588. While it is my opinion that Quake discloses a fluorinated surfactant comprising an oligoethylene glycol, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $F-(CF_2)_i-(CH_2CH_2-O)_j-H$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $FC_{7.5}E_8$) and Zonyl FSN-100 (approximately $FC_{8.2}E_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

3589. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 10*

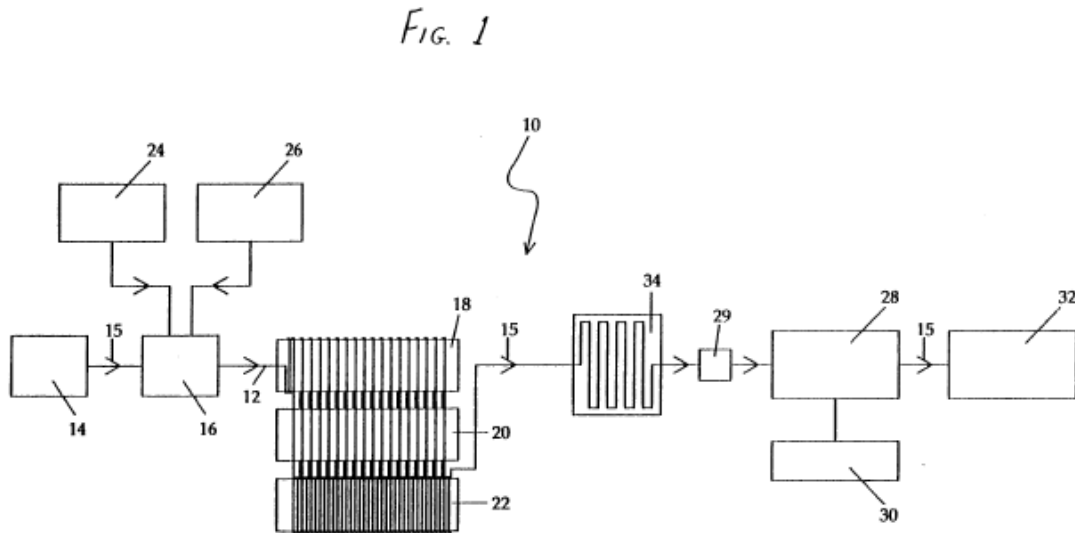
3590. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3591. Claim 10 further recites: **“the at least one plug contains at least one reagent for an autocatalytic reaction.”**

3592. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the '083 patent explains, PCR is a type of autocatalytic reaction. *See* '083 patent at 44:36-39 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

3593. While it is my opinion that Quake discloses that at least one plug contains at least one reagent for an autocatalytic reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase),

oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μl , is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3594. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v)

hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3595. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3596. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior

Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

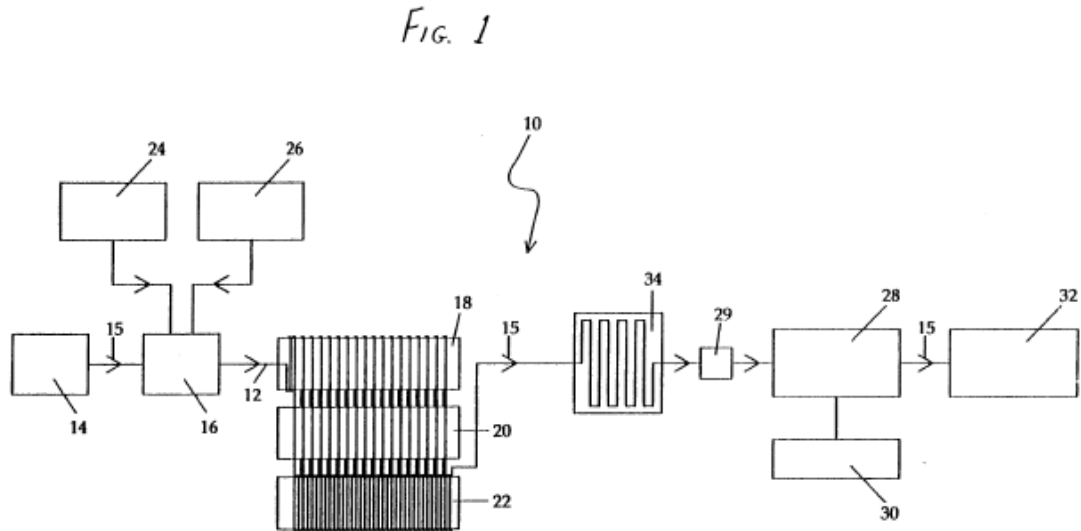
3597. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference my analysis with respect to claims 1 and 10.

3598. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

3599. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added).

3600. While it is my opinion that Quake discloses a polymerase-chain reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume,

approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3601. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole

orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3602. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3603. It also would have been obvious that the at least one plug contain at least one reagent for a polymerase-chain reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of

Success), and all references cited therein.

(vi) *Claim 12*

3604. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3605. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

3606. Quake satisfies this limitation. For example, Quake discloses that “[i]n preferred embodiments, the droplets of aqueous solution have a volume of approximately 0.1 to 100 picoliters (pL).” Quake at [0003].

3607. Quake also explains that “[f]or particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are preferably rounded, with a diameter between 2 and 100 microns, preferably about 60 microns, and more preferably about 30 microns at the crossflow area or droplet extrusion region . . . Similarly, the volume of the detection region in an analysis device is typically in the range of between about 10 femtoliters (fL) and 5000 fL, preferably about 40 or 50 fL to about 1000 or 2000 fL, most preferably on the order of about 200 fL. In preferred embodiments, the channels of the device, and particularly the channels of the inlet connecting to a droplet extrusion region, are between about 2 and 50 microns, most preferably about 30 microns.” Quake at [0091].

3608. Quake also provides a formula that can be used to calculate droplet size:

The size of a droplet in a micro fluidic device of this invention may be provided by the equation:

$$r = \frac{\sigma}{\eta \epsilon}$$

where r is the final droplet radius in a main channel. η , the viscosity of the continuous phase (e.g., the oil-surfactant phase in the above exemplary devices) and σ , the interfacial tension, may be obtained from values available in the art for the particular fluids used (see, for example, CRC Handbook of Chemistry and Physics, CRC Press, Inc., Boca Raton, Fla., 2000). ε , which denotes the shear rate, may be provided by the formula

$$\varepsilon = \frac{2}{y_0} v,$$

where v is the velocity of the dispersed phase fluid (i.e., the droplets) and may be readily calibrated to the input pressures for a particular microfluidic device. Y_0 denotes the radius of the inlet channel at the droplet extrusion region (i.e., the radius of the tapered channel 1606 in FIG. 16B).

Quake at [0308]-[0310].

(vii) *Claim 13*

3609. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3610. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

3611. Quake satisfies this limitation. For example, Quake states that “[c]hannels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, or from silicon-urethane composites such as those available from Polymer Technology Group (Berkeley, Calif.), e.g., PurSil™ and CarboSil™.” Quake at [0118]. Quake also discloses that “[i]n a preferred embodiment, the invention provides a “T” on “Y” shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS.” Quake at [0216]. A POSA would have understood that silicon elastomer and PDMS are both

polymers.

(viii) *Claim 20*

3612. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

3613. I understand that the Court has not considered whether the preamble of this claim is limiting.

3614. Regardless of whether the preamble is limiting, Quake satisfies this claim limitation. For example, the abstract in Quake describes the inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

3615. Quake describes that, in some embodiments, the droplets created in the microfluidic device may be used as “microreactors”: “For instance, *in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions)* or are used to analyze and/or sort biochemical, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Quake at [0095] (emphasis added).

3616. Quake even describes a specific type of chemical reaction involving enzymes produced by cells:

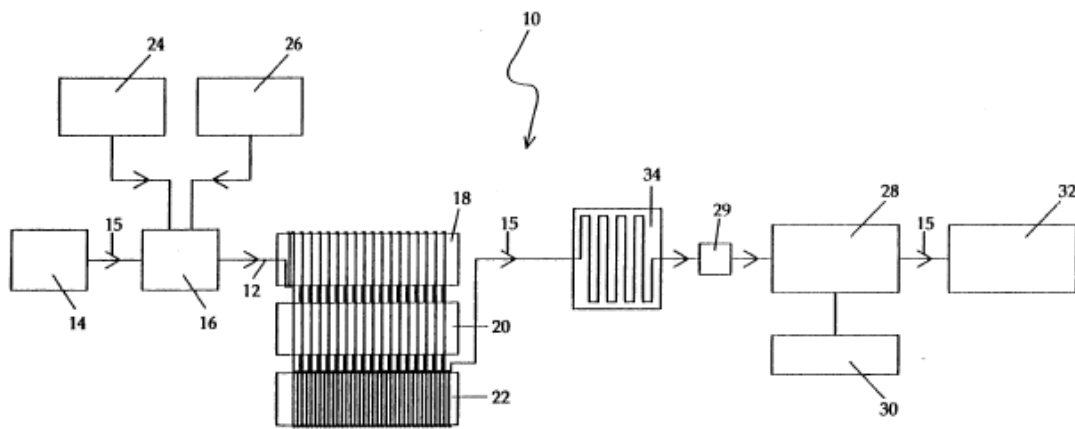
In another embodiment, *cells may produce a reporter in vivo (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change.* This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For

example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate.

Quake at [0170] (emphasis added).

3617. While it is my opinion that Quake discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3618. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3619. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3620. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3621. It also would have been obvious to conduct a reaction within at least one plug based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3622. Claim 20 further recites: “**introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.**”

3623. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention,

which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3624. For example, Quake also describes that “[i]n preferred embodiments, *a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device* and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” Quake at [0020] (emphasis added); *see also* Quake at [0113] (emphasis added) (“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (*preferably a non-polar fluid such as decane or other oil*) in the main channel.”).

3625. Quake further described experimental testing using oils. For example, in Example 12, Quake disclosed that “[v]arious oils were tested in the devices, including decane, tetradecane and hexadecane.” Quake at [0300].

3626. Quake also discloses a non-fluorinated microchannel. For example, Quake states that “[c]hannels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, of from silicon-urethane composites such as those available from Polymer Technology Group (Berkeley, Calif.), e.g., PurSil™ and CarboSil™.” Quake at [0118]. Quake also discloses that “[i]n a preferred embodiment, the invention provides a “T” on “Y” shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS.” Quake at [0216].

3627. Quake also discloses fluorinated oils and fluorinated surfactants. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span,

fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added).

3628. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

3629. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Quake. West at 2324. Therefore, a POSA would have understood that Quake’s disclosure of fluorinated surfactants included fluorinated surfactants with hydrophilic headgroups.

3630. While it is my opinion that Quake discloses a fluorinated oil and fluorinated surfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications”

and “are chemically and biologically stable.” *Id.*

3631. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3632. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3633. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3634. Claim 20 further recites: “**introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms**

in the carrier-fluid after the at least one stream contacts the carrier-fluid.”

3635. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize *small droplets of aqueous solution within microfluidic channels filled with oil*. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel*, e.g., at an angle that is perpendicular to the stream of oil. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that *the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream thereby forming droplets.*” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”).

3636. For example, Quake also describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” Quake at [0020] (emphasis added).

3637. Quake also made clear that his patent application described the forming of droplets by partitioning aqueous fluid with carrier fluid. For example, during prosecution of his

patent application, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Quake ’103 Response at 15.

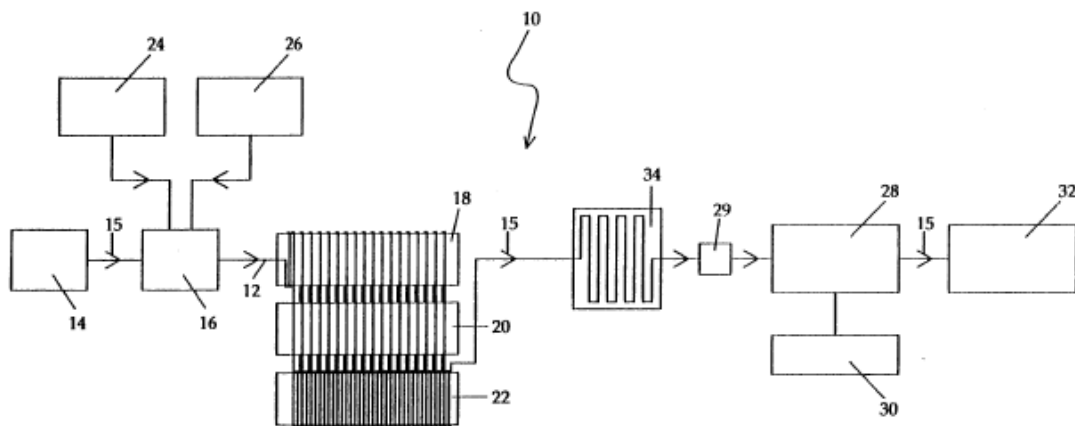
3638. Claim 20 further recites: “**wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.**”

3639. Quake satisfies this limitation. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR*.” Quake at [0080] (emphasis added). As the ’083 patent explains, PCR is a type of autocatalytic reaction. *See* ’083 patent at 44:36-39 (“Another example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

3640. While it is my opinion that Quake discloses an aqueous fluid and at least one reagent for an autocatalytic reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the

DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3641. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally,

“[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3642. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3643. It also would have been obvious to use an aqueous fluid and at least one reagent

for an autocatalytic reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3644. Claim 20 further recites: “**the at least one plug-fluid is immiscible with the carrier-fluid.**”

3645. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0015] (“A first fluid flows through the main channel, and *a second fluid, which is incompatible or immiscible with the [first] fluid*, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3646. Claim 20 further recites: “**each plug is substantially surrounded on all sides by carrier-fluid.**”

3647. Quake satisfies this limitation. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis

added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

3648. Claim 20 further recites: “**and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

3649. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added). Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion.

3650. In 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well

defined droplet size distribution. . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3651. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid.

3652. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous. Quake described that “silicon elastomer (RTV) channels, which are hydrophobic and advantageously do not absorb water, . . . may tend to swell when exposed to an oil phase . . . Urethane substrates do not tend to swell in oil but are hydrophilic, they may undesirably absorb water, and tend to use higher operating pressures [to ensure the oil flow rate sustains an encapsulating film of carrier oil].” Quake at [0118]. A POSA would have understood that a hydrophilic urethane surface could enable droplet contact with the wall, if the oil flow rate was not high enough. Quake further states that “the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol/vol) of either 0.5, 1.0 or 2.0%. The devices were equilibrated prior to crossflow by priming the out flow channel with oil to eliminate interactions of the aqueous [plug] phase with the hydrophilic urethane walls of the channel.” Quake at [0300]. Therefore, Quake teaches this limitation.

3653. While it is my opinion that Quake discloses a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3654. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3655. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 21*

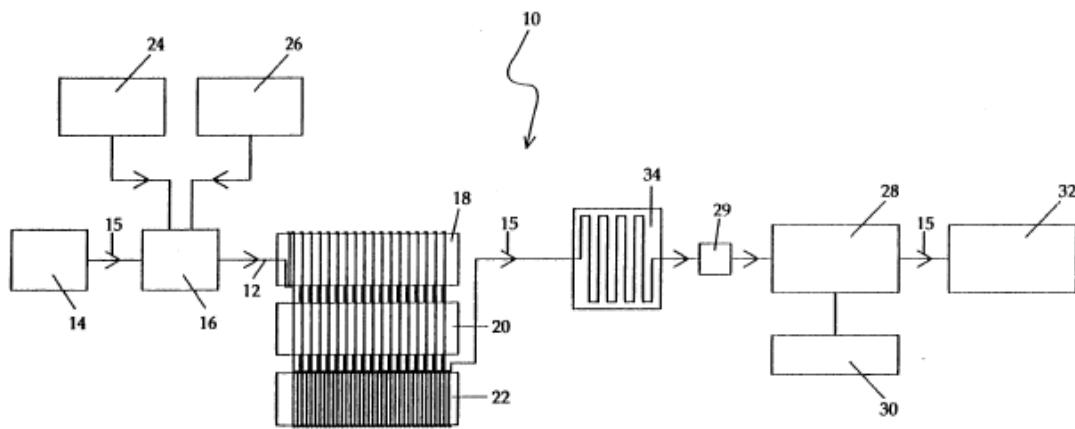
3656. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3657. Claim 21 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

3658. Quake satisfies this element. For example, Quake discloses that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, *such as PCR.*” Quake at [0080] (emphasis added).

3659. While it is my opinion that Quake discloses a polymerase-chain reaction, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3660. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3661. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3662. It also would have been obvious to conduct a polymerase-chain reaction based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

3663. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3664. Claim 22 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

3665. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*”).

3666. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

3667. While it is my opinion that Quake discloses a carrier-fluid comprising a fluorinated compound, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3668. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3669. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3670. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

3671. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

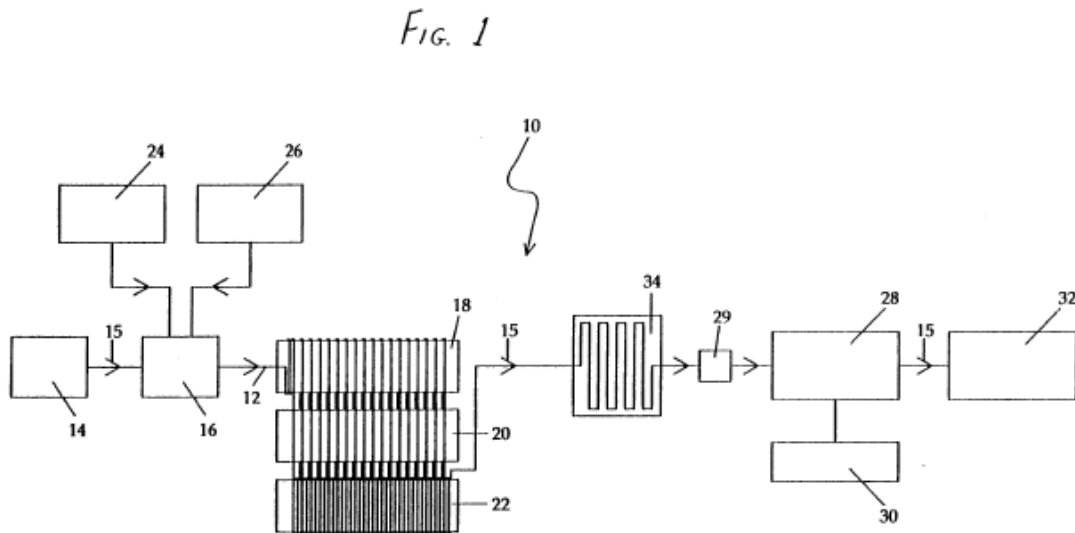
3672. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a**

virion, an enzyme, DNA and RNA.”

3673. Quake satisfies this limitation. For example, Quake describes that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the *biological material or sample*.” Quake at [0020] (emphasis added). Quake further describes that “[i]n various embodiments of the method, the biological material may be, e.g., *molecules (for example, polynucleotides, polypeptides, enzymes, substrates, or mixtures thereof), cells or viral particles*, or mixtures thereof.” Quake at [0021] (emphasis added). Quake defines “polynucleotide” as including “*double and single stranded RNA and DNA*.” Quake at [0052] (emphasis added).

3674. While it is my opinion that Quake discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active

fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

3675. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole

orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3676. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3677. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 31*

3678. Claim 31 recites: “**A microfluidic system.**”

3679. Quake satisfies this limitation. For example, the abstract in Quake describes the

inventions in the patent application as “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided.” Quake at Abstract.

3680. Claim 31 further recites: “**a non-fluorinated microchannel.**”

3681. Quake satisfies this limitation. For example, Quake states that “[c]hannels of the invention may be formed from silicon elastomer (e.g. RTV), urethane compositions, of from silicon-urethane composites such as those available from Polymer Technology Group (Berkeley, Calif.), e.g., PurSil™ and CarboSil™.” Quake at [0118]. Quake also discloses that “[i]n a preferred embodiment, the invention provides a “T” on “Y” shaped series of channels molded into optically transparent silicone rubber or PolyDiMethylSiloxane (PDMS), preferably PDMS.” Quake at [0216].

3682. Claim 31 further recites: “**a fluorinated carrier fluid.**”

3683. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which *a pressurized stream of oil is passed*, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003] (emphasis added); *see also* Quake at [0287] (emphasis added) (“[I]n preferred embodiments the device partitions droplets of an aqueous solution, which typically contains a sample of molecules or particles (e.g., cells or virions) into a *pressurized stream or flow of oil in a main channel of the device.*”); Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid,

passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3684. Quake also describes that the carrier fluid may be fluorinated. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane*.”).

3685. I understand that the parties’ agreed-to construction for “fluorinated oil” is “an oil that includes one or more fluorine atoms.” Quake describes that the fluids of his invention, including the oil acting as a carrier fluid, “may contain additives,” including “fluorinated oils.” An oil—even an unfluorinated oil, such as a mineral oil—containing a fluorinated oil as an additive would fall within this construction of “fluorinated oil,” as an oil that includes one or more fluorine atoms.

3686. While it is my opinion that Quake discloses a fluorinated carrier fluid, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil,

and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3687. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3688. It also would have been obvious to use a fluorinated carrier fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3689. It also would have been obvious to use a fluorinated carrier fluid based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3690. Claim 31 further recites: “**a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.**”

3691. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added); *see also* Quake at [0118] (emphasis added) (“The channels may also be coated with additives or agents, such as surfactants, TEFLON, or *fluorinated oils such as octadecafluorooctane (98%, Aldrich) or fluorononane.*”).

3692. Quake also states that the carrier fluid, or “extrusion fluid,” may contain surfactants. For example, Quake discloses that “[a]n extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel The extrusion fluid may also contain one or more additives, *such as surfactants*” Quake at [0022] (emphasis added); *see also* Quake at [0020] (“[I]n preferred embodiments the extrusion fluid is a non-polar solvent or oil (e.g., decane, tetradecane, or hexadecane) and contains at least one surfactant.”); Quake at [0096] (“In one particularly preferred embodiment, the extrusion fluid is an oil (e.g., decane, tetradecane, or hexadecane) that contains a surfactant (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%).”). Quake describes the “sample fluid” as the aqueous fluid “containing the biological material for analysis, reaction or sorting” Quake at [0020].

3693. A POSA would further understand that surfactants with a hydrophobic tail and a

hydrophilic head were most commonly used with oil-water systems, such as the systems described in Quake. West at 2324. Therefore, a POSA would have understood that Quake's disclosure of fluorinated surfactants included fluorinated surfactants with hydrophilic headgroups.

3694. While it is my opinion that Quake discloses a fluorinated surfactant, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes "[m]icroemulsions containing fluorinated oil" prepared by "mixing water, the perfluorinated oil, and a fluorinated surfactant." Schubert at 97. Schubert notes that "[f]luorinated compounds . . . offer the potential for biomedical applications" and "are chemically and biologically stable." *Id.*

3695. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A "dispersing agent" or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these "fluorochemical emulsions" have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3696. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3697. Claim 31 further recites: **"and at least one plug comprising an aqueous plug-**

fluid in the microchannel and substantially encased by the carrier-fluid.”

3698. Quake satisfies this limitation. For example, Quake describes the devices and methods disclosed in his patent application as “designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” Quake at [0003]; *see also* Quake at [0015] (“A first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the [first] fluid, passes through the inlet region so that the droplets of the second fluid are sheared into the main channel.”); Quake at [0070] (emphasis added) (“An ‘extrusion region’ or ‘droplet extrusion region’ is a junction between an inlet region and the main channel of a chip of the invention, which permits the introduction of a pressurized fluid to the main channel at an angle perpendicular to the flow of fluid in the main channel.”).

3699. Quake also discloses that the droplets are substantially encased by the carrier fluid. For example, Quake discloses that “[i]n embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous *droplets are encapsulated or separated by each other by oil.*” Quake at [0100] (emphasis added); *see also* Quake at [0241] (emphasis added) (“In the case of water-in-oil micelle . . . a differential in the index of refraction between two phases of a droplet system, e.g., *where droplets of one phase are separated or encapsulated by another phase*, may be exploited to move or direct droplets in response to radiation pressure.”).

3700. Claim 31 further recites: “**wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

3701. Quake satisfies this limitation. For example, Quake describes that “[t]he fluids used in the invention may contain additives, such as *agents which reduce surface tensions (surfactants)*. Exemplary surfactants include Tween, Span, *fluorinated oils*, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel.” Quake at [0117] (emphasis added). Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion.

3702. In 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3703. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the

plug-fluid or carrier fluid.

3704. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous. Quake described that “silicon elastomer (RTV) channels, which are hydrophobic and advantageously do not absorb water, . . . may tend to swell when exposed to an oil phase . . . Urethane substrates do not tend to swell in oil but are hydrophilic, they may undesirably absorb water, and tend to use higher operating pressures [to ensure the oil flow rate sustains an encapsulating film of carrier oil].” Quake at [0118]. A POSA would have understood that a hydrophilic urethane surface could enable droplet contact with the wall, if the oil flow rate was not high enough. Quake further states that “the oil phase introduced into the device also contained a surfactant (Span 80) with concentrations (vol/vol) of either 0.5, 1.0 or 2.0%. The devices were equilibrated prior to crossflow by priming the out flow channel with oil to eliminate interactions of the aqueous [plug] phase with the hydrophilic urethane walls of the channel.” Quake at [0300]. Therefore, Quake teaches this limitation.

3705. While it is my opinion that Quake discloses a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface, it also would have been obvious to combine the teachings of Quake with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert

notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3706. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3707. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Quake in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(b) Invalidity Based on Shaw Stewart

3708. It is my opinion that Shaw Stewart discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

3709. Claim 1 recites: “**A microfluidic system.**”

3710. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or

preparing mixtures of reagents” Shaw Stewart at 1:7-9. Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

3711. Shaw Stewart II further discloses that the system is “particularly suited to the manipulation of microscopic quantities of reactant with volumes of less than 10 nanolitres.” Shaw Stewart II at 3:18-19. As Bio-Rad has admitted, at least Shaw Stewart II “discloses a ‘microfluidic product.’” IPR2015-00009 Petition at 8.

3712. Claim 1 further recites: “**a non-fluorinated microchannel.**”

3713. Shaw Stewart satisfies this limitation. For example, Shaw Stewart states that “[t]he preferred [sic] apparatus for carrying out the method comprises *a plastic block* (2) with indentations (3) which is clamped against *a glass plate* (4) forming closed conduits which are connected to reservoirs of light mineral oil, or silicon oil, by thin tubing (9) and valves (10).” Shaw Stewart at Abstract (emphasis added). The numbers in the description correspond to Figure 8 of Shaw Stewart, reproduced below:

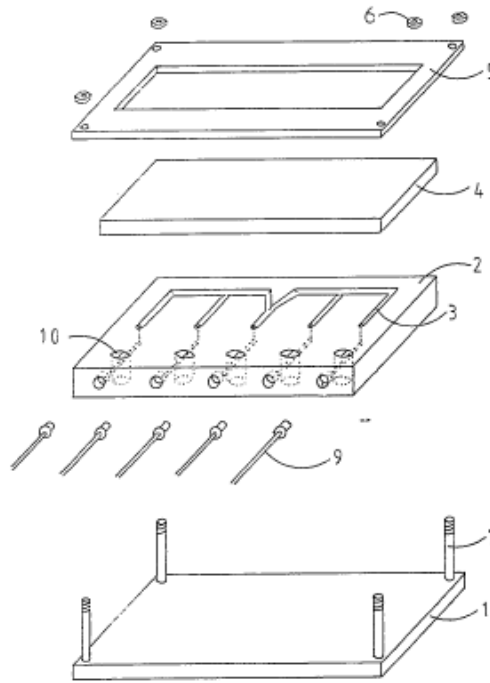


Figure 8.

Shaw Stewart at Fig. 8. Shaw Stewart also described that “[f]or aqueous reagents, *glass tubing* and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66 (emphasis added).

3714. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel.**”

3715. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two

currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

3716. Shaw Stewart II explicitly describes that the carrier fluid can be a fluorinated oil. Shaw Stewart II at 4 (emphasis added) (“Suitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.”).

3717. Shaw Stewart also discloses a surfactant. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging. Suitable carrier phases include cholesterol, sodium dioxyol, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

3718. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with

microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3719. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3720. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3721. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of

Success), and all references cited therein.

3722. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Shaw Stewart. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

3723. Claim 1 further recites: **“at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

3724. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart discloses this limitation.

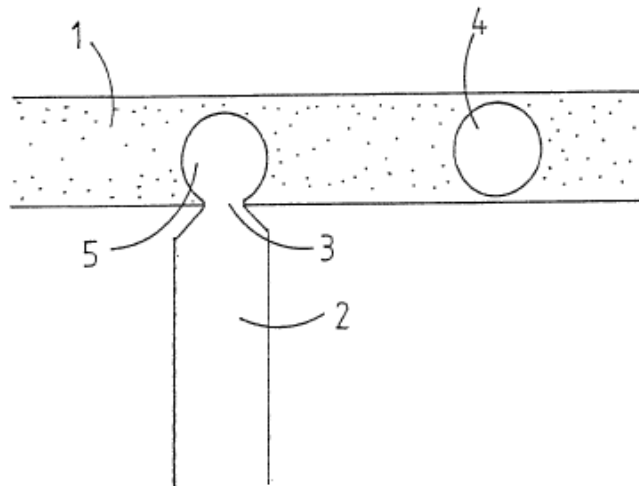


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75. Shaw Stewart described that the “reagent” could refer to “aqueous reagents,” stating that “[f]or aqueous reagents, glass

tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66.

3725. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

3726. Claim 1 further recites: **“wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

3727. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3728. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3729. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid] interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3730. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3731. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher

than surface tension at the plug-fluid/carrier fluid interface based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

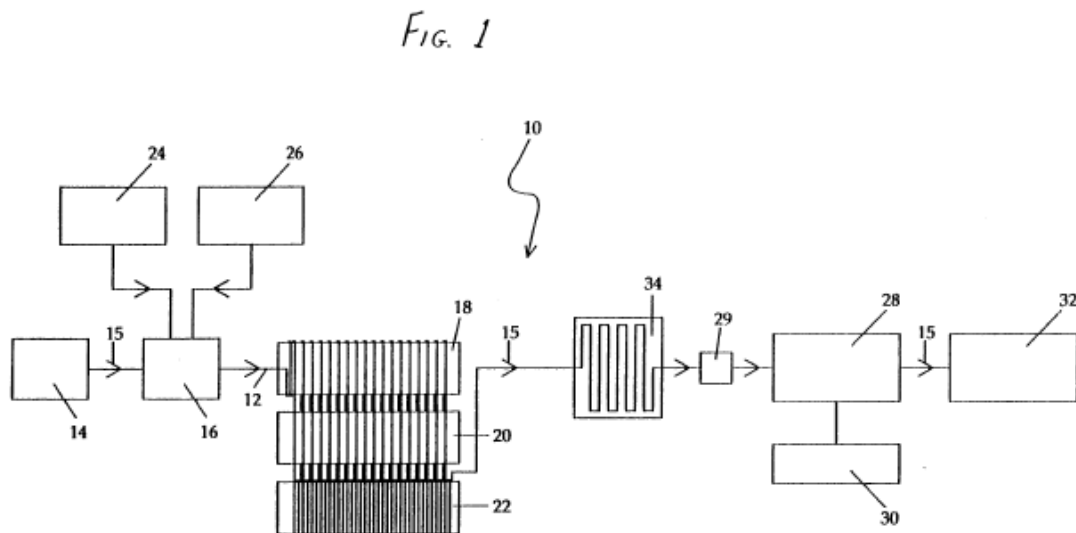
3732. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3733. Claim 2 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.**”

3734. Shaw Stewart satisfies this limitation. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added).

3735. While it is my opinion that Shaw Stewart discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or

more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

3736. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these

assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3737. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3738. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background

Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 9*

3739. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3740. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

3741. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3585-3589, demonstrating how Quake discloses that at least one plug comprises an oligoethylene glycol.

3742. As another example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $\text{F}-(\text{CF}_2)_i-(\text{CH}_2\text{CH}_2\text{-O})_j\text{-H}$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $\text{FC}_{7.5}\text{E}_8$) and Zonyl FSN-100 (approximately $\text{FC}_{8.2}\text{E}_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

3743. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 10*

3744. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

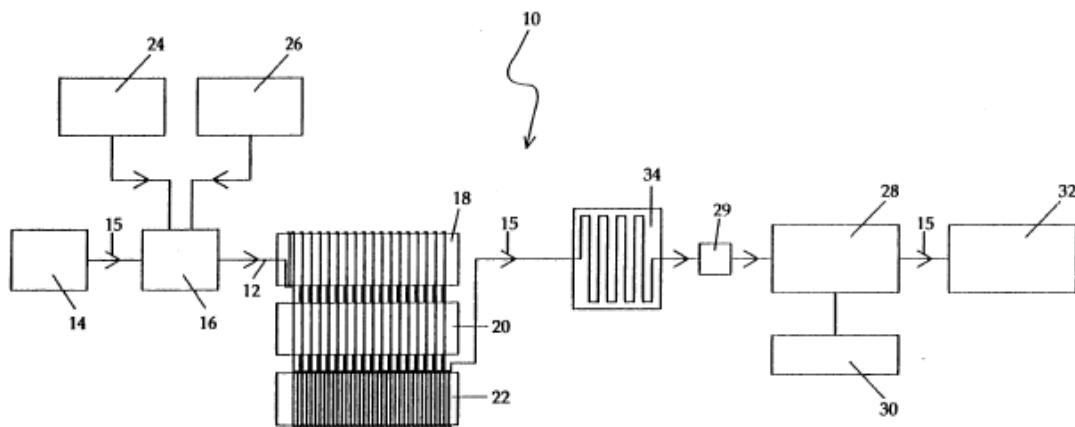
3745. Claim 10 further recites: **“the at least one plug contains at least one reagent for an autocatalytic reaction.”**

3746. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added).

3747. While it is my opinion that Shaw Stewart discloses that at least one plug contains at least one reagent for an autocatalytic reaction, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture

comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3748. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these

assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3749. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3750. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

3751. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference my analysis with respect to claims 1 and 10.

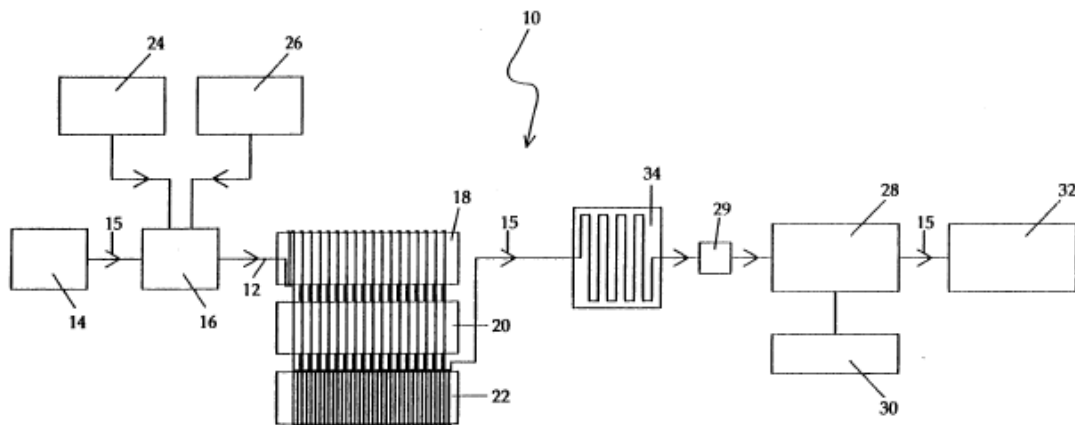
3752. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

3753. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added).

3754. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3755. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE)

analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3756. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR

amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3757. It also would have been obvious that the at least one plug contain at least one reagent for a polymerase-chain reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 12*

3758. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3759. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

3760. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3604-3608, demonstrating how Quake discloses that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters.

3761. It also would have been obvious that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 13*

3762. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3763. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

3764. Shaw Stewart satisfies this limitation. For example, Shaw Stewart states that “[t]he preferred [sic] apparatus for carrying out the method comprises *a plastic block* (2) with indentations (3) which is clamped against *a glass plate* (4) forming closed conduits which are connected to reservoirs of light mineral oil, or silicon oil, by thin tubing (9) and valves (10).” Shaw Stewart at Abstract (emphasis added). The numbers in the description correspond to Figure 8 of Shaw Stewart, reproduced below:

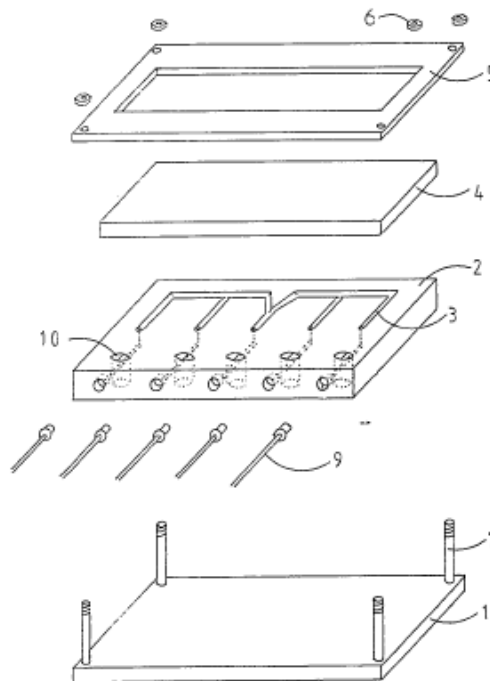


Figure 8.

Shaw Stewart at Fig. 8. Shaw Stewart also described that “[f]or aqueous reagents, *glass tubing*

and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66 (emphasis added).

(viii) *Claim 20*

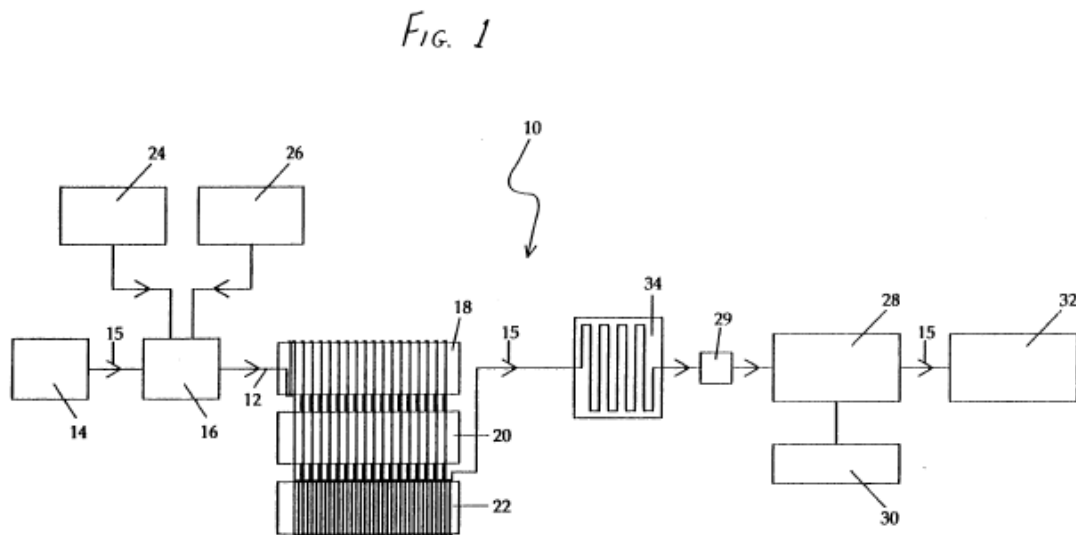
3765. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

3766. I understand that the Court has not considered whether the preamble of this claim is limiting.

3767. Regardless of whether the preamble is limiting, Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

3768. While it is my opinion that Shaw Stewart discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an

enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3769. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for

operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3770. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3771. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or

nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3772. It also would have been obvious to conduct a reaction within at least one plug based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3773. Claim 20 further recites: **“introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.”**

3774. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes that “if large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while *a continuous current of carrier phase flows down the tube*. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart continuously refers to a carrier fluid, stating that the “reagent liquid, hereafter referred to as a reagent, will be supported

and moved by another, immiscible liquid, referred to hereafter as the carrier phase.” Shaw Stewart at 1:36-39. Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicons, or Freons.” Shaw Stewart at 1:39-41 (emphasis added). Further, Shaw Stewart II explicitly describes that the carrier fluid can be a fluorinated oil. Shaw Stewart II at 4 (emphasis added) (“Suitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.”).

3775. Figure 1 of Shaw Stewart also discloses this limitation.

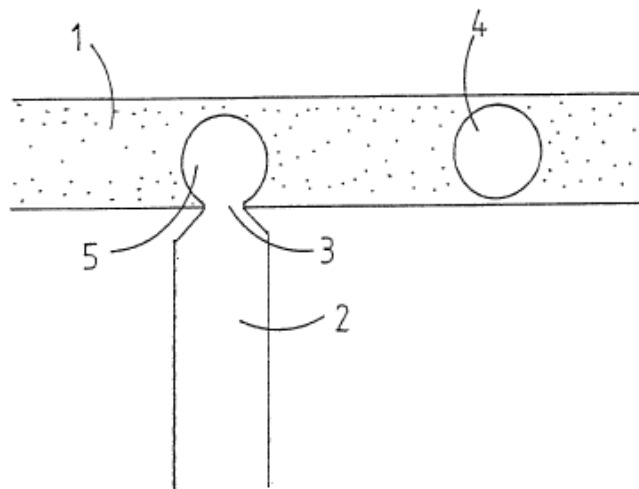


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

3776. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad

stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

3777. Shaw Stewart also discloses a non-fluorinated microchannel. For example, Shaw Stewart states that “[t]he preferred [sic] apparatus for carrying out the method comprises *a plastic block* (2) with indentations (3) which is clamped against *a glass plate* (4) forming closed conduits which are connected to reservoirs of light mineral oil, or silicon oil, by thin tubing (9) and valves (10).” Shaw Stewart at Abstract (emphasis added).

3778. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing

fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3779. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3780. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3781. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3782. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Shaw Stewart. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

3783. Claim 20 further recites: **“introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid.”**

3784. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that “if large numbers of droplets are required, *a continuous flow of reagent* through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” Shaw Stewart at 1:83-90 (emphasis added). Shaw Stewart described that this “continuous flow of reagent” could refer to aqueous solution, stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66. Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86.

3785. Figure 1 of Shaw Stewart also discloses this limitation.

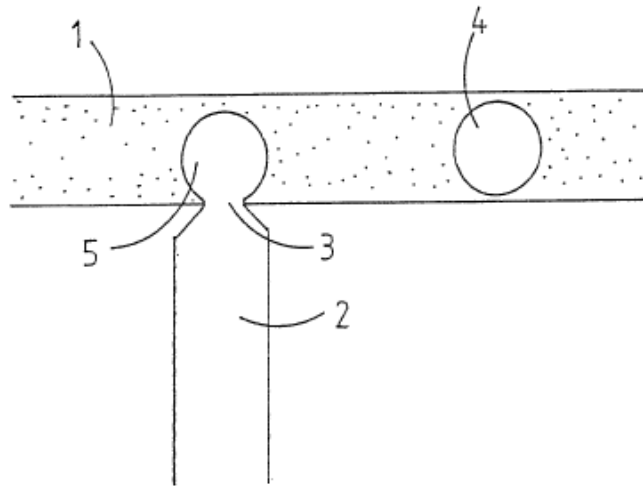


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

3786. Further, plaintiff Bio-Rad has recently took the (correct) position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. In an IPR filed against a Quake patent, Bio-Rad stated that:

To overcome Stewart I [“Shaw Stewart”] and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a ‘stepwise fashion,’ whereas in the ’503 Patent droplets are formed by ‘combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.’ In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an

immiscible fluid:

If large numbers of droplets are required a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

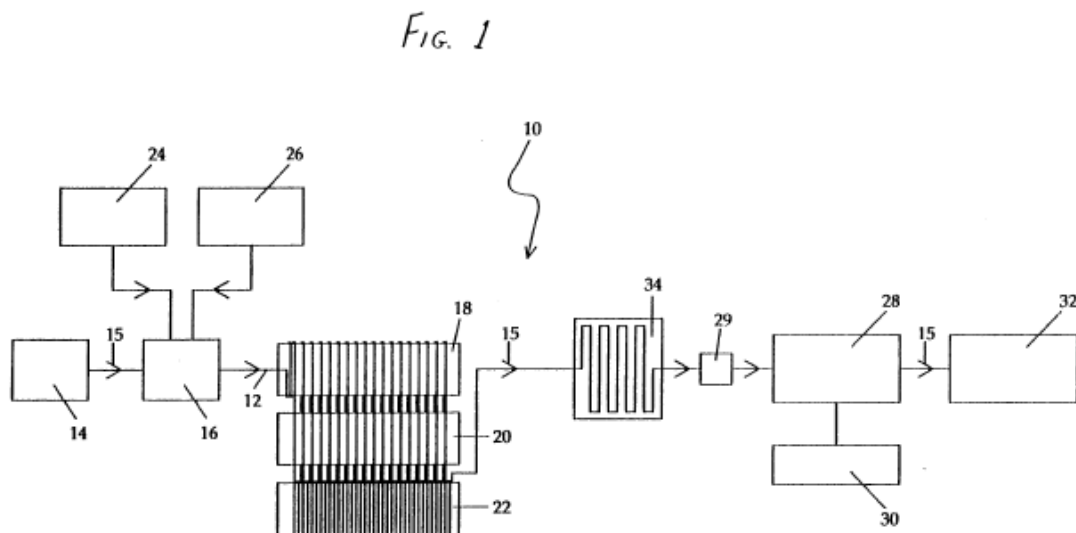
IPR2015-00009 Petition at 2-3. Therefore, plaintiff and exclusive licensee Bio-Rad has explicitly argued that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

3787. Claim 20 further recites: **“wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.”**

3788. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for *initiating and controlling a chemical reaction or preparing mixtures of reagents . . .*” Shaw Stewart at 1:7-9 (emphasis added). Shaw Stewart also describes that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.” Shaw Stewart at 3:82-86. Shaw Stewart also described that the aqueous sample solution could include “liquids containing suspended biological micro-organisms.” Shaw Stewart at 4:30-33.

3789. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction

mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3790. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR

amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3791. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to

a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3792. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3793. Claim 20 further recites: “**the at least one plug-fluid is immiscible with the carrier-fluid.**”

3794. Shaw Stewart satisfies this limitation. For example, Shaw Stewart describes that the “reagent liquid, hereafter referred to as a reagent, will be supported and moved by *another, immiscible liquid, referred to hereafter as the carrier phase.*” Shaw Stewart at 1:36-39 (emphasis added). Shaw Stewart also described the carrier fluid as an “inert immiscible liquid,” that separated “discrete volume[s].” Shaw Stewart at 1:11-14. Shaw Stewart also disclosed that “[s]uitable carrier phases include *mineral oils*, water, light silicones, or Freons.” Shaw Stewart at 1:39-41 (emphasis added).

3795. Claim 20 further recites: “**each plug is substantially surrounded on all sides by carrier-fluid.**”

3796. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart discloses this limitation.

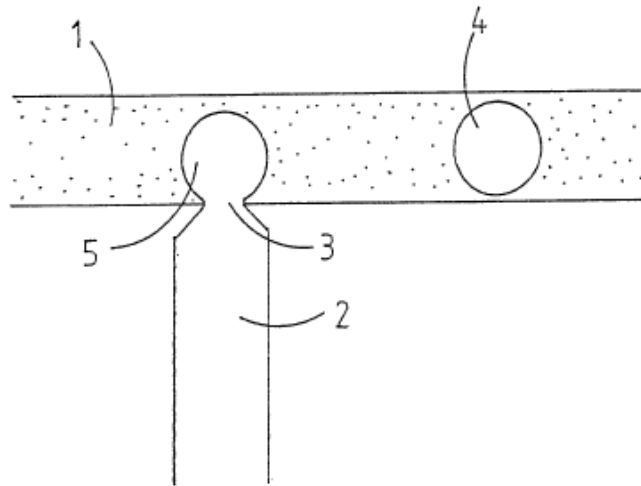


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75.

3797. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

3798. Claim 20 further recites: **“and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

3799. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes

“[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3800. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3801. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension . . . If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3802. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous

drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3803. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 21*

3804. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

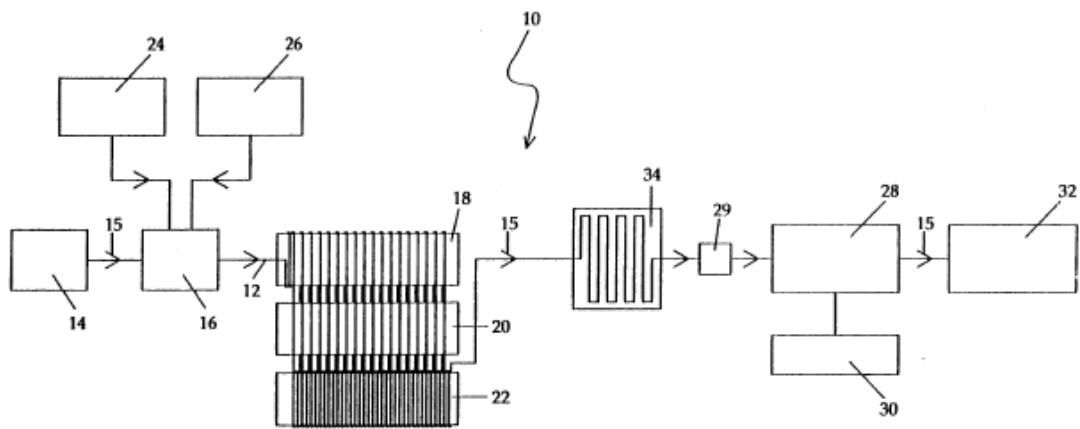
3805. Claim 21 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

3806. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended *biological micro-organisms*.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, *biochemistry*, geology, etc., especially in procedures which utilize very

small quantities, such as forensic and *recombinant DNA work*.” Shaw Stewart at 3:82-86 (emphasis added).

3807. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3808. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3809. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3810. It also would have been obvious to conduct a polymerase-chain reaction based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

3811. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3812. Claim 22 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

3813. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons*.” Shaw Stewart II at 4 (emphasis added).

3814. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3815. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3816. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes,

and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3817. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

3818. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

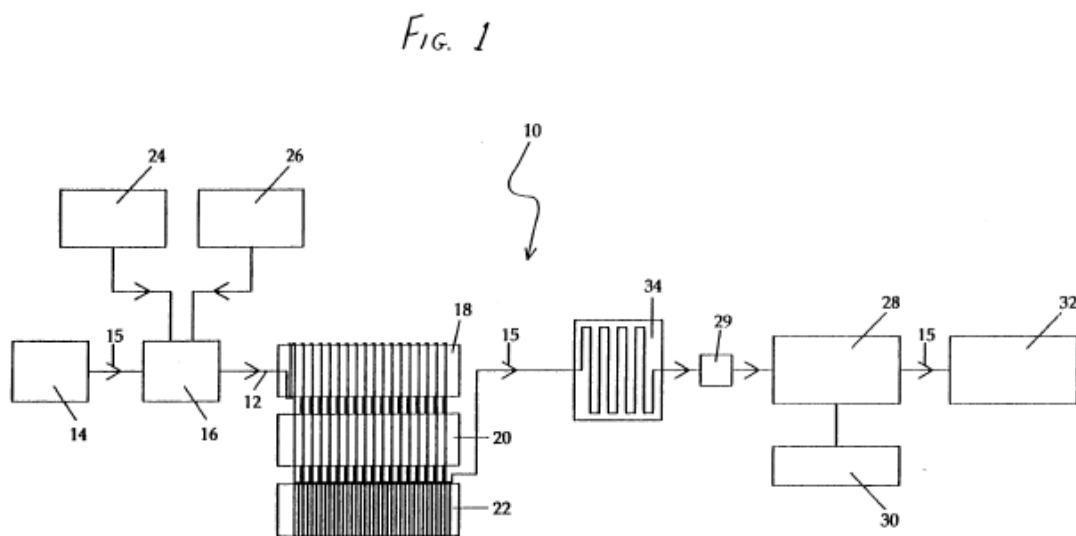
3819. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.**”

3820. Shaw Stewart satisfies this limitation. For example, Shaw Stewart described that the reagent-containing aqueous solution could include “liquids containing suspended **biological micro-organisms**.” Shaw Stewart at 4:30-33 (emphasis added). Shaw Stewart also described that “[t]his invention “may have applications in many branches of medicine, chemistry, **biochemistry**, geology, etc., especially in procedures which utilize very small quantities, such as forensic and **recombinant DNA work**.” Shaw Stewart at 3:82-86 (emphasis added).

3821. While it is my opinion that Shaw Stewart discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to

combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

3822. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3823. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops

through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3824. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 31*

3825. Claim 31 recites: “**A microfluidic system.**”

3826. Shaw Stewart satisfies this limitation. For example, Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents” Shaw Stewart at 1:7-9. Shaw Stewart also described that the system described was a microfluidic system, disclosing that “[t]he system is particularly suited to the manipulation of *microscopic quantities of reagents*, with volumes of less than one microliter” Shaw Stewart at 1:20-22 (emphasis added).

3827. Shaw Stewart II further discloses that the system is “particularly suited to the manipulation of microscopic quantities of reactant with volumes of less than 10 nanolitres.” Shaw Stewart II at 3:18-19. As Bio-Rad has admitted, at least Shaw Stewart II “discloses a ‘microfluidic product.’” IPR2015-00009 Petition at 8.

3828. Claim 31 further recites: “**a non-fluorinated microchannel.**”

3829. Shaw Stewart satisfies this limitation. For example, Shaw Stewart states that “[t]he preferred [sic] apparatus for carrying out the method comprises *a plastic block* (2) with

indentations (3) which is clamped against *a glass plate* (4) forming closed conduits which are connected to reservoirs of light mineral oil, or silicon oil, by thin tubing (9) and valves (10).” Shaw Stewart at Abstract (emphasis added). The numbers in the description correspond to Figure 8 of Shaw Stewart, reproduced below:

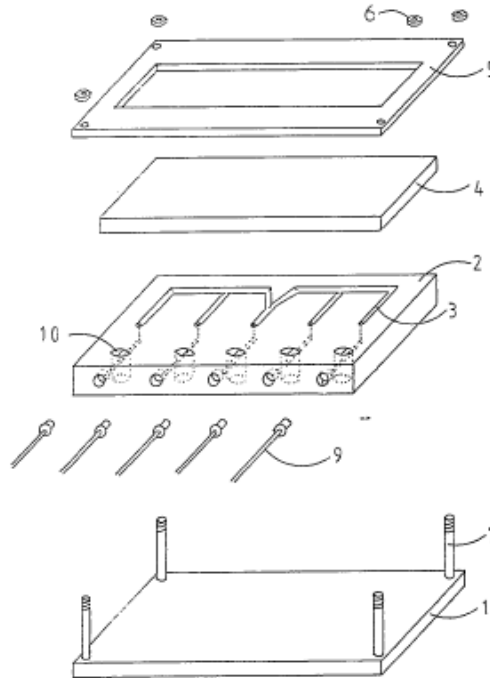


Figure 8.

Shaw Stewart at Fig. 8. Shaw Stewart also described that “[f]or aqueous reagents, *glass tubing* and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66 (emphasis added).

3830. Claim 31 further recites: “**a fluorinated carrier fluid.**”

3831. Shaw Stewart II satisfies this limitation. For example, Shaw Stewart II discloses that “[s]uitable carrier phases include mineral oils, light silicon oils, water, and *fluorinated hydrocarbons.*” Shaw Stewart II at 4 (emphasis added).

3832. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart states that “[s]uitable carrier phases include mineral oils, water, light silicones, or Freons.” Shaw Stewart at 1:39-41.

3833. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3834. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3835. It also would have been obvious to use a fluorinated carrier fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been

used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3836. It also would have been obvious to use a fluorinated carrier fluid based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3837. Claim 31 further recites: “**a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.**”

3838. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Shaw Stewart describes “surface acting chemical agents” can be dissolved “in the immiscible liquid.” Shaw Stewart at 4:26-29. Shaw Stewart further discloses that “[s]urface acting agents *may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.* Suitable carrier phases include cholesterol, sodium dioyl, succinate Teepol, and Triton-X-100.” Shaw Stewart at 1:44-48 (emphasis added); *see also* Shaw Stewart at 2:19-26 (emphasis added) (“It is convenient to use a carrier phase for carrying the droplets to the U-tube which contains *a surfacting agent* which prevents merging, and to introduce a small quantity of immiscible carrier phase containing a surfacting agent which encourages merging by means of a side arm, which the droplets are in position in the U-tube.”).

3839. It also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious

to use a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3840. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3841. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3842. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Shaw Stewart. West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

3843. Claim 31 further recites: **“and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

3844. Shaw Stewart satisfies this limitation. For example, Figure 1 of Shaw Stewart discloses this limitation.

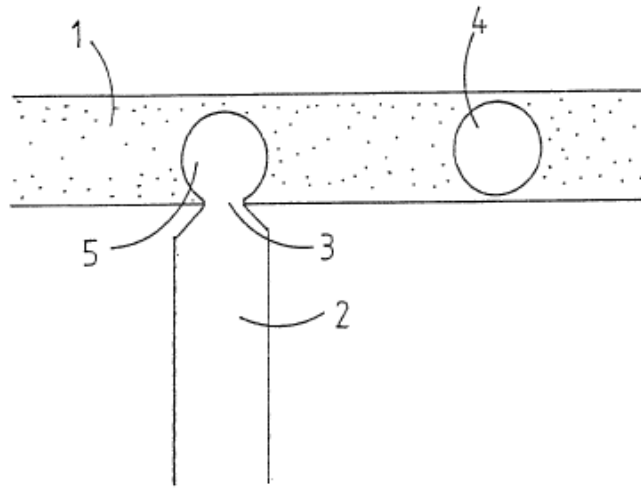


Figure 1.

Shaw Stewart at Fig. 1. As described in Shaw Stewart, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Shaw Stewart at 1:70-75. Shaw Stewart described that the “reagent” could refer to “aqueous reagents,” stating that “[f]or aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Shaw Stewart at 1:62-66.

3845. Shaw Stewart also states that the method it claims involves “discrete volumes of chemical reagents [that] are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.” Shaw Stewart at 3:102-104.

3846. Claim 31 further recites: “**wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is**

higher than surface tension at the plug-fluid/carrier fluid interface.”

3847. Shaw Stewart at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Shaw Stewart with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3848. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3849. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interfacel tension . . . If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . .

[to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3850. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3851. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Shaw Stewart in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(c) Invalidity Based on Burns (2001)

3852. It is my opinion that Burns (2001) discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon

which they depend.

(i) *Claim 1*

3853. Claim 1 recites: “**A microfluidic system.**”

3854. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel has been developed. The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a large enhancement in the interfacial mass transfer and the reaction rate. Mass transfer performance data has been obtained for a *glass chip-based reactor in a 380 μm wide channel* by monitoring the extraction of acetic acid from kerosene slugs as they moved along the reactor channel.” Burns (2001) at Abstract (emphasis added).

3855. Figure 4 from Burns (2001) also illustrates a microfluidic system:

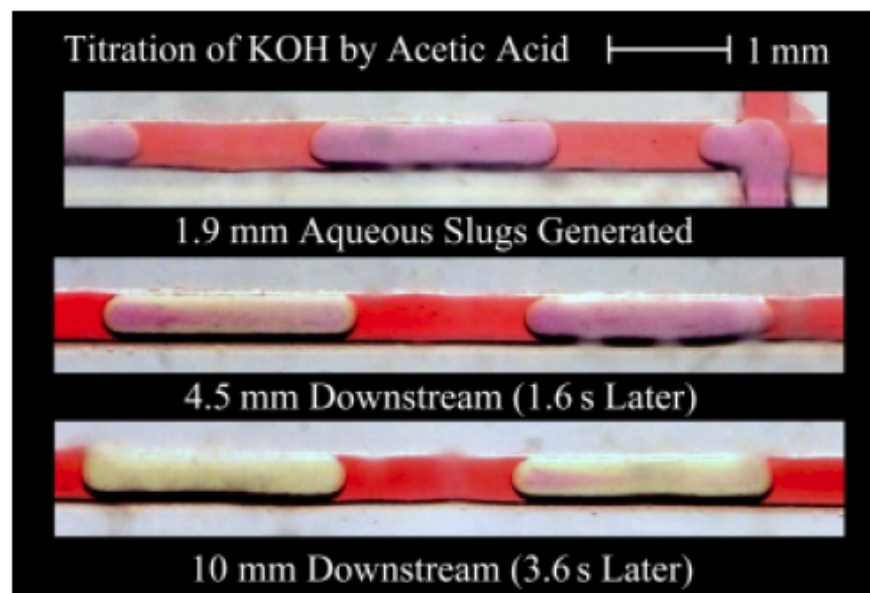


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3856. Claim 1 further recites: “**a non-fluorinated microchannel.**”

3857. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that the microfluidic chip used is made of soda-lime glass: “All of the experiments reported here were performed using a soda-lime glass device. Channels were cut into the device using a 380 μm thick slitting saw. The depth of the channels was chosen to match their width, which was approximately 380 μm .” Burns (2001) at 11.

3858. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel.**”

3859. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

3860. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1

to 0.4 mol l^{-1} . *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l^{-1} solutions.” Burns (2001) at 11 (emphasis added).

3861. This reaction is illustrated in Figure 4:

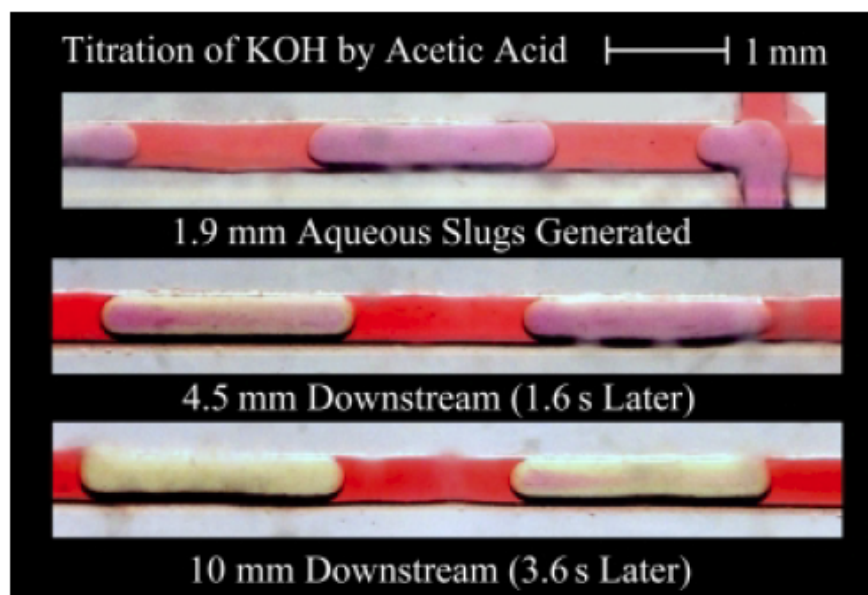


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3862. It also would have been obvious to combine the teachings of Burns with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3863. It also would have been obvious to use a fluorinated oil and fluorinated surfactant

with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3864. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3865. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3866. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the system described in Burns (2001). West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated

surfactants with hydrophilic headgroups.

3867. Claim 1 further recites: “**at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.**”

3868. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11.

3869. Figure 4 also illustrates this process:

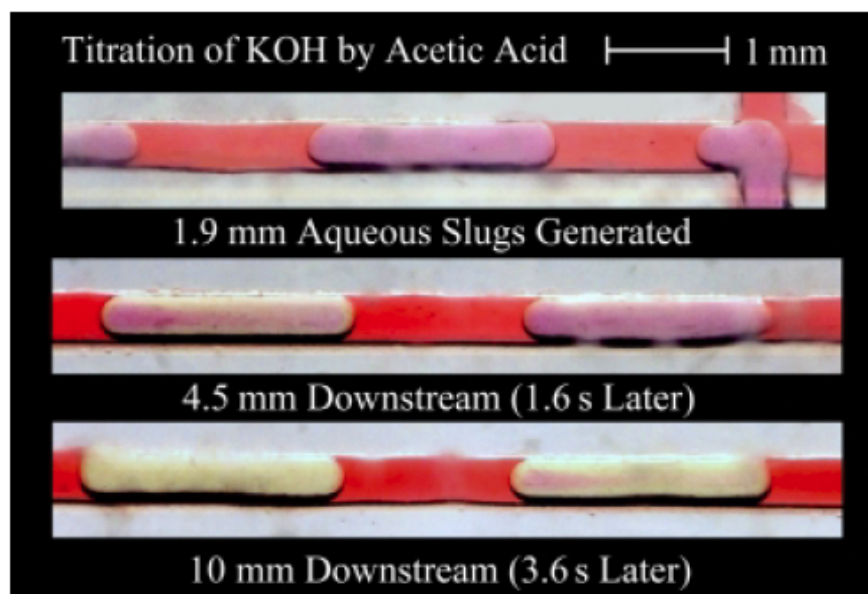


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4. Figure 4 also demonstrates that each “slug” is substantially surrounded by immiscible carrier fluid.

3870. When a less viscous fluid moves as a “plug” through a more viscous fluid in a

micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

3871. Claim 1 further recites: **“wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

3872. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3873. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract.

Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3874. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3875. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic

channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3876. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

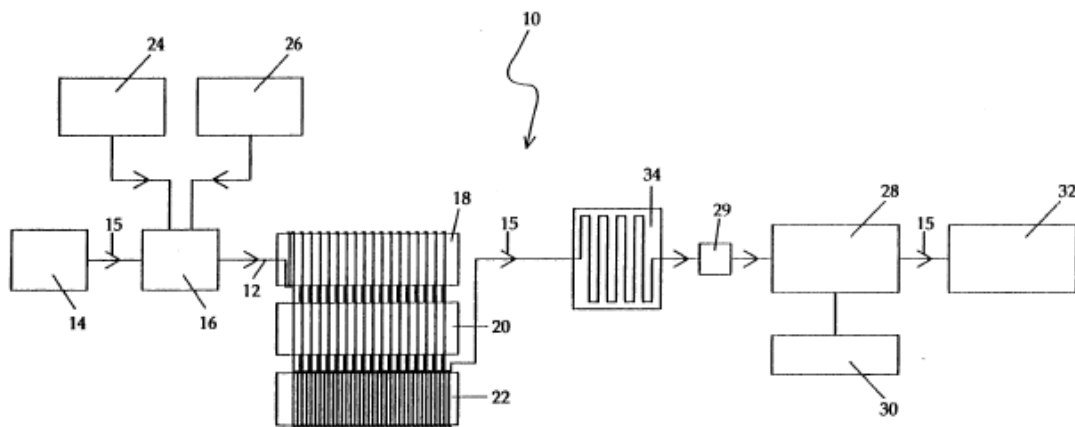
3877. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3878. Claim 2 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.**”

3879. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

3880. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3881. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3882. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 9*

3883. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3884. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

3885. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3585-3589, demonstrating how Quake discloses that at least one plug comprises an oligoethylene glycol.

3886. As another example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $F-(CF_2)_i-(CH_2CH_2-O)_j-H$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $FC_{7.5}E_8$) and Zonyl FSN-100 (approximately $FC_{8.2}E_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

3887. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 10*

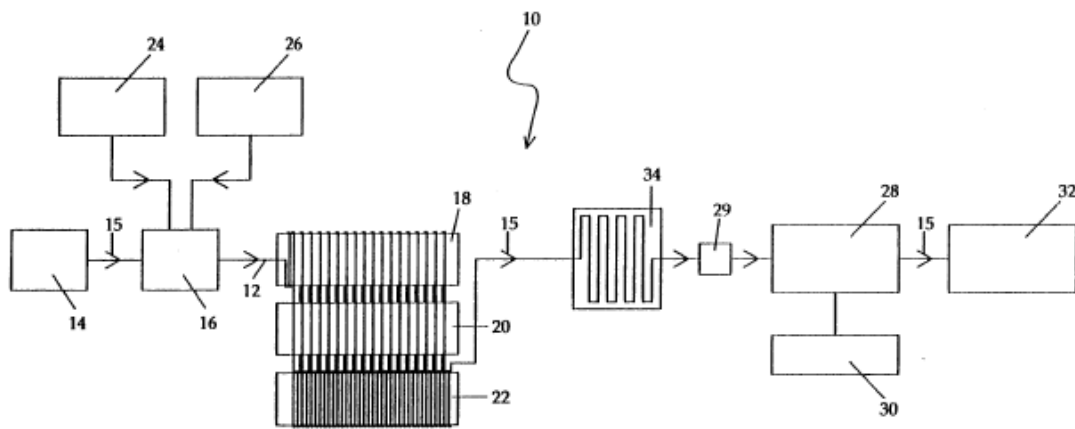
3888. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

3889. Claim 10 further recites: **“the at least one plug contains at least one reagent for an autocatalytic reaction.”**

3890. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3891. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3892. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3893. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

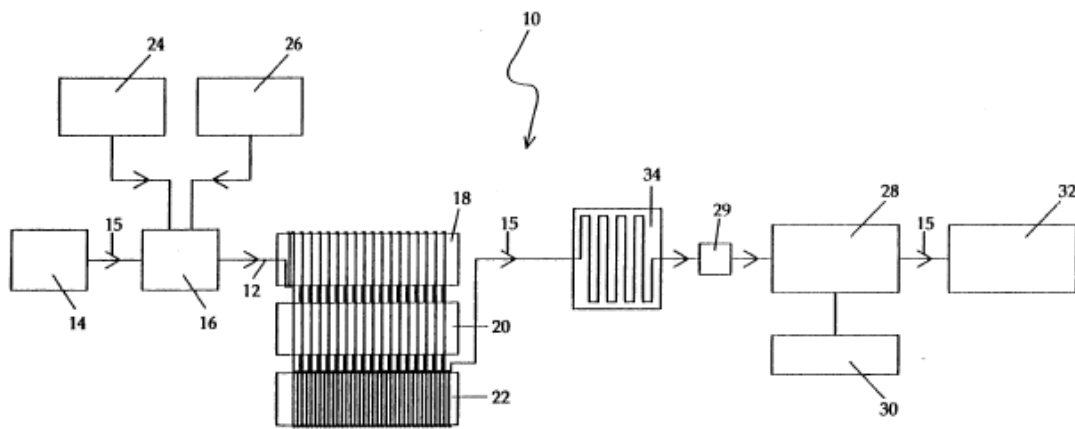
3894. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference

my analysis with respect to claims 1 and 10.

3895. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

3896. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3897. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3898. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3899. It also would have been obvious that the at least one plug contain at least one reagent for a polymerase-chain reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 12*

3900. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

3901. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

3902. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3604-3608, demonstrating how Quake discloses that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters.

3903. It also would have been obvious that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 13*

3904. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

3905. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

3906. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that the microfluidic chip used is made of soda-lime glass: “All of the experiments reported here were performed using a soda-lime glass device. Channels were cut into the device using a 380 μm thick slitting saw. The depth of the channels was chosen to match their width, which was approximately 380 μm .” Burns (2001) at 11.

(viii) *Claim 20*

3907. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

3908. I understand that the Court has not considered whether the preamble of this claim is limiting.

3909. Regardless of whether the preamble is limiting, Burns (2001) satisfies this claim limitation. For example, Burns (2001) discloses “[a] *multiphase microreactor* based upon the use of slug flow through a narrow channel has been developed.” Burns (2001) at Abstract (emphasis added); *see also* Burns (2001) at 14 (“The mass transfer results from this study indicate that slug flow offers a viable alternative for reacting two phase flow within a micro-channel environment.”).

3910. Burns (2001) also describes the specific reaction conducted: A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. Kerosene was used as the basis of the organic phase with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11.

3911. This reaction is illustrated in Figure 4:

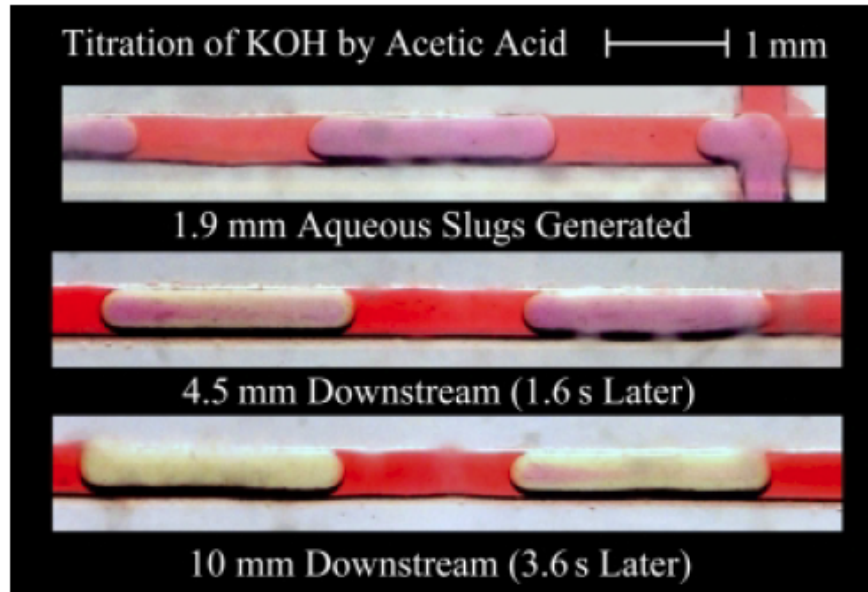
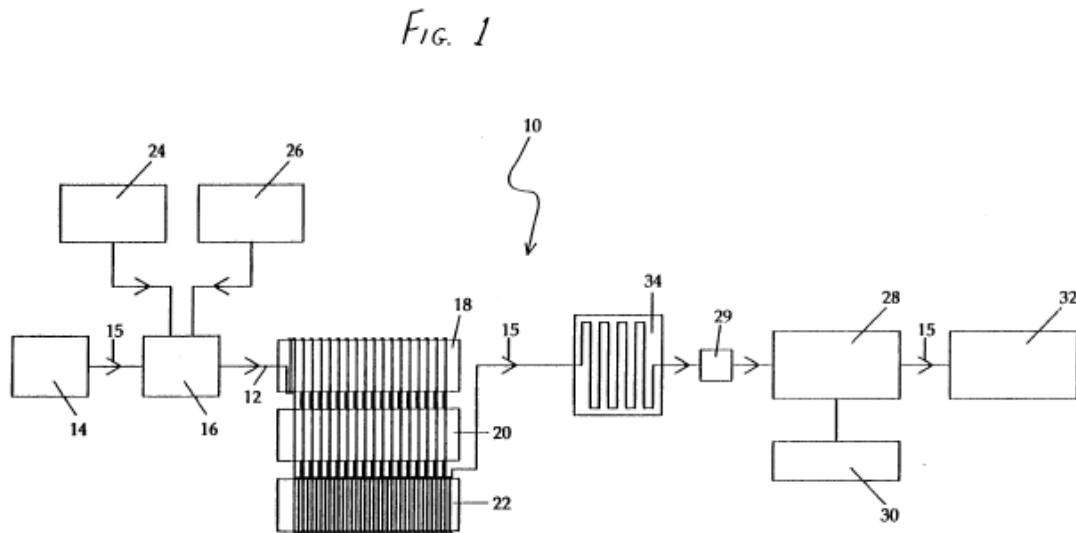


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3912. While it is my opinion that Burns (2001) discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into

tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3913. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from

the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3914. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3915. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may

react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

3916. It also would have been obvious to conduct a reaction within at least one plug based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3917. Claim 20 further recites: **“introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.”**

3918. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. *Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection*, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

3919. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the

diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

3920. This reaction is illustrated in Figure 4:

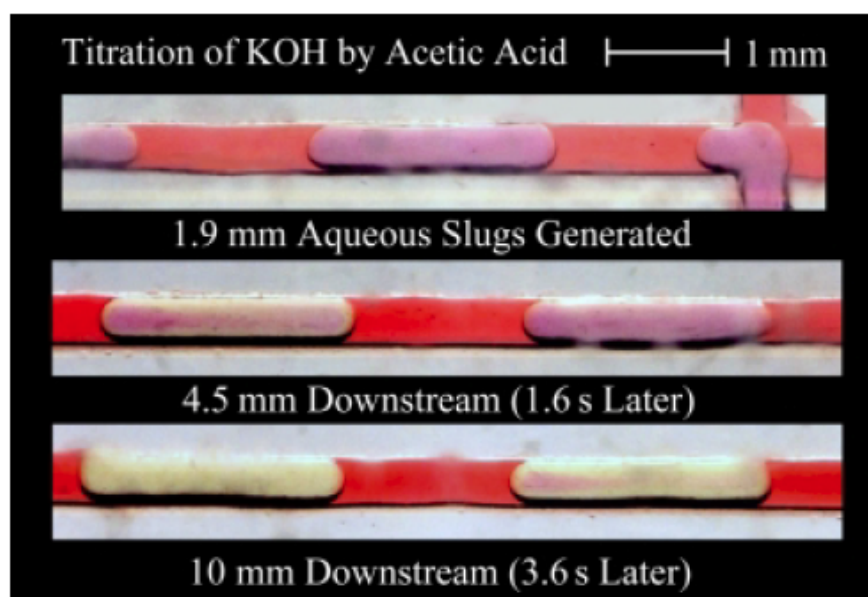


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3921. Burns (2001) also discloses a non-fluorinated microchannel. For example, Burns (2001) describes that the microfluidic chip used is made of soda-lime glass: “All of the experiments reported here were performed using a soda-lime glass device. Channels were cut

into the device using a 380 μm thick slitting saw. The depth of the channels was chosen to match their width, which was approximately 380 μm .” Burns (2001) at 11.

3922. Burns (2001) also discloses a non-fluorinated microchannel. For example, Burns (2001) describes that the microfluidic chip used is made of soda-lime glass: “All of the experiments reported here were performed using a soda-lime glass device. Channels were cut into the device using a 380 μm thick slitting saw. The depth of the channels was chosen to match their width, which was approximately 380 μm .” Burns (2001) at 11.

3923. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3924. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3925. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for

carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3926. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3927. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the system described in Burns. West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

3928. Claim 20 further recites: **“introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid.”**

3929. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the

other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11.

3930. Figure 4 also illustrates this process:

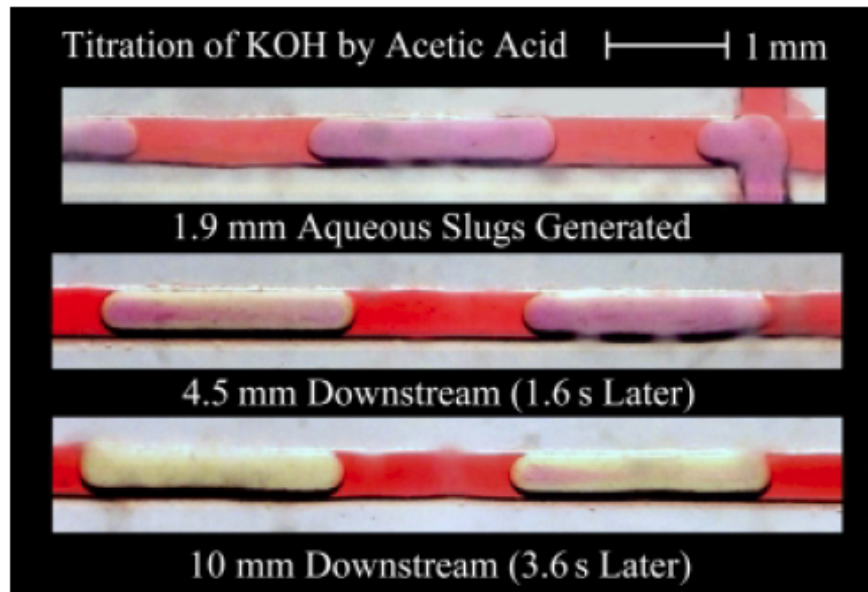


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

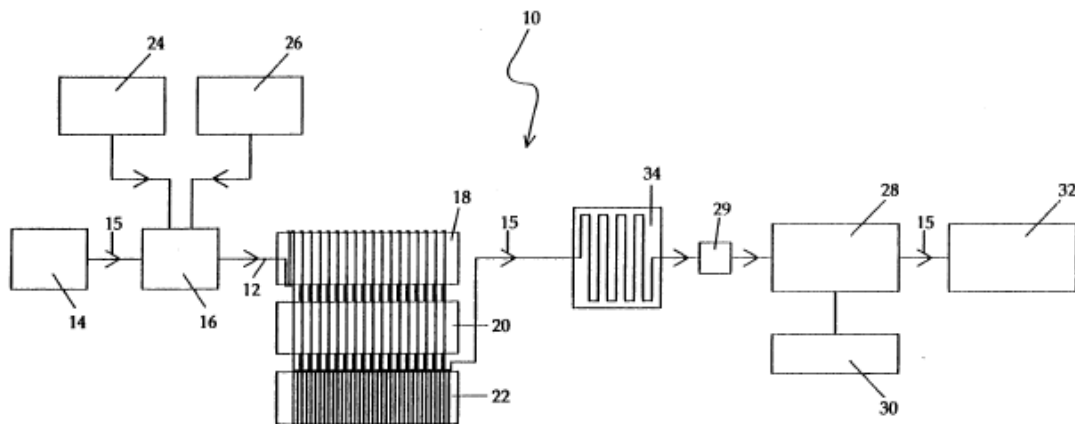
Burns (2001) at Fig. 4.

3931. Claim 20 further recites: “**wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.**”

3932. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture

passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3933. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR

amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3934. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to

a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3935. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3936. Claim 20 further recites: “**the at least one plug-fluid is immiscible with the carrier-fluid.**”

3937. Burns (2001) satisfies this limitation. For example, in explaining the specific reaction conducted, Burns (2001) discloses that the “slugs” are comprised of aqueous fluid, separated and surrounded by kerosene, an oil immiscible with the aqueous fluid: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce *aqueous solutions of KOH and NaOH* in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis

added).

3938. Claim 20 further recites: “**each plug is substantially surrounded on all sides by carrier-fluid.**”

3939. Burns (2001) satisfies this limitation. For example, Figure 4 shows that each “slug” is substantially surrounded by immiscible carrier fluid:

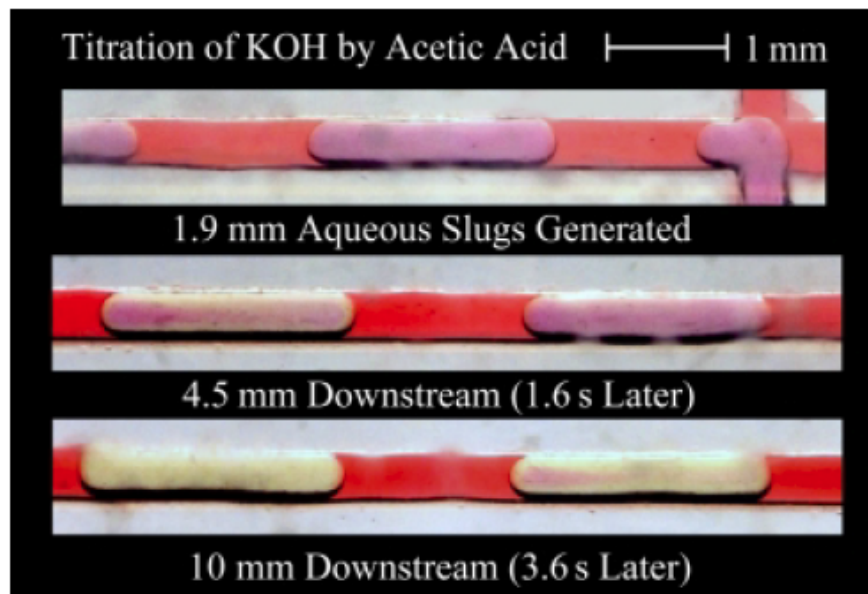


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3940. When a less viscous fluid moves as a “plug” through a more viscous fluid in a micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these

“slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

3941. Claim 20 further recites: **“and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

3942. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3943. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3944. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-

fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid] interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . . “). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3945. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3946. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and

Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

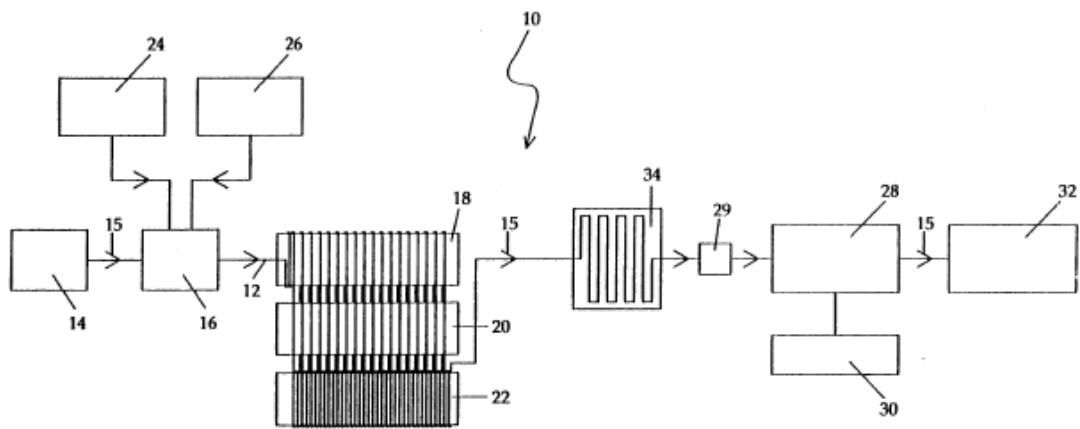
(ix) *Claim 21*

3947. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3948. Claim 21 further recites: **“the autocatalytic reaction is a polymerase-chain reaction.”**

3949. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3950. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3951. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

3952. It also would have been obvious to conduct a polymerase-chain reaction based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

3953. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3954. Claim 22 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

3955. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3956. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3957. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because

they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” Id. at 6:46-50.

3958. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

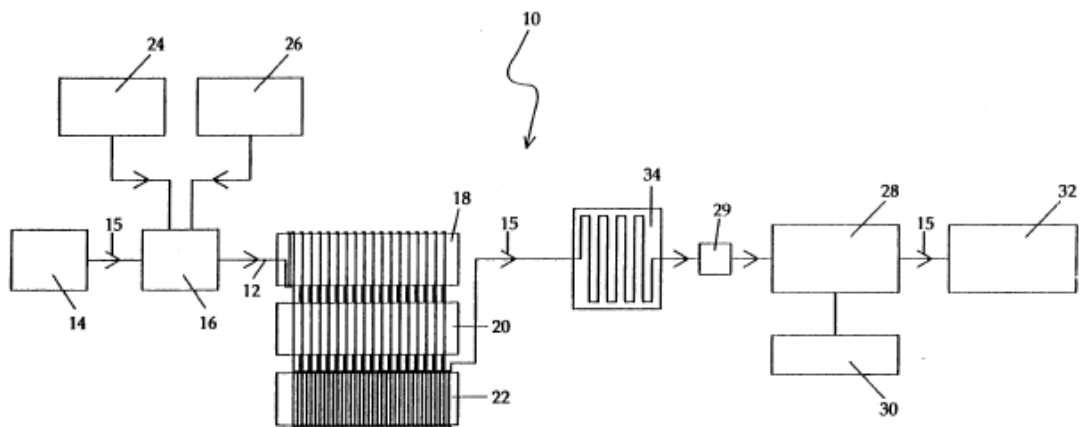
3959. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

3960. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.**”

3961. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

3962. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

3963. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

3964. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3965. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 31*

3966. Claim 31 recites: “**A microfluidic system.**”

3967. Burns (2001) satisfies this limitation. For example, Burns (2001) discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel has been developed. The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a large enhancement in the interfacial mass transfer and the reaction rate. Mass transfer performance data has been obtained for a *glass chip-based reactor in a 380 μm wide channel* by monitoring the extraction of acetic acid from kerosene slugs as they moved along the reactor channel.” Burns (2001) at Abstract (emphasis added).

3968. Figure 4 from Burns (2001) also illustrates a microfluidic system:

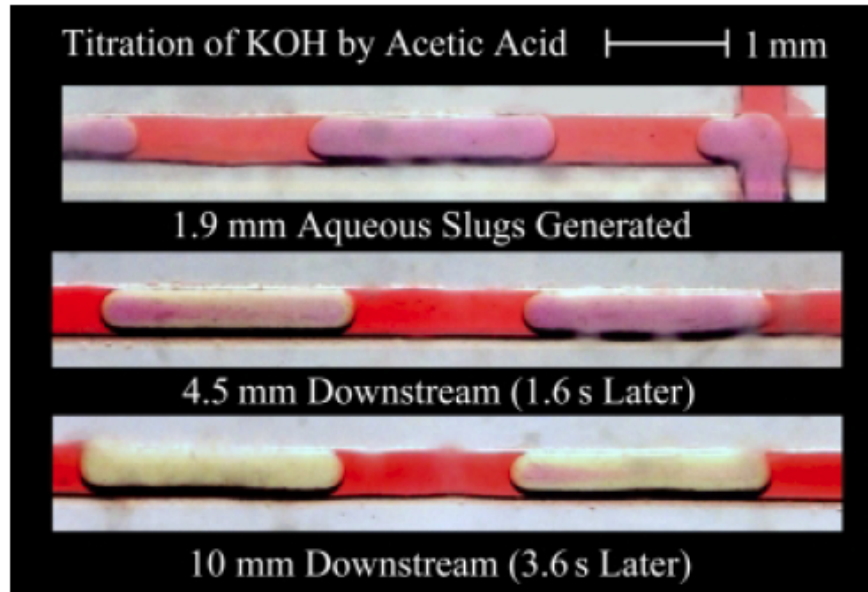


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3969. Claim 31 further recites: “**a non-fluorinated microchannel.**”

3970. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that the microfluidic chip used is made of soda-lime glass: “All of the experiments reported here were performed using a soda-lime glass device. Channels were cut into the device using a 380 μm thick slitting saw. The depth of the channels was chosen to match their width, which was approximately 380 μm .” Burns (2001) at 11.

3971. Claim 31 further recites: “**a fluorinated carrier fluid.**”

3972. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method discussed in this work uses *the continuous flow of both phases* through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing

into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11 (emphasis added).

3973. Burns (2001) also makes clear that carrier fluid immiscible with the aqueous fluid is used to form slugs. For example, Burns (2001) discloses: “A simple acid–base reaction was used as the basis of this study because, being extremely rapid, its progress was controlled by the diffusion of the reactants within the slugs. It had the further advantage that conventional pH indicators could be used to follow the reaction progress. These also clarified the photographic distinction between the phases. Phenol Red pH indicator was added to distilled water to create a saturated system, which was filtered and then diluted to by a factor of two with more distilled water. This was then used to produce aqueous solutions of KOH and NaOH in the range from 0.1 to 0.4 mol l⁻¹. *Kerosene was used as the basis of the organic phase* with Sudan III (red) or Sudan IV (blue) dye added to aid flow visualisation. Acetic acid was then added to the kerosene to produce 0.5 and 0.65 mol l⁻¹ solutions.” Burns (2001) at 11 (emphasis added).

3974. This reaction is illustrated in Figure 4:

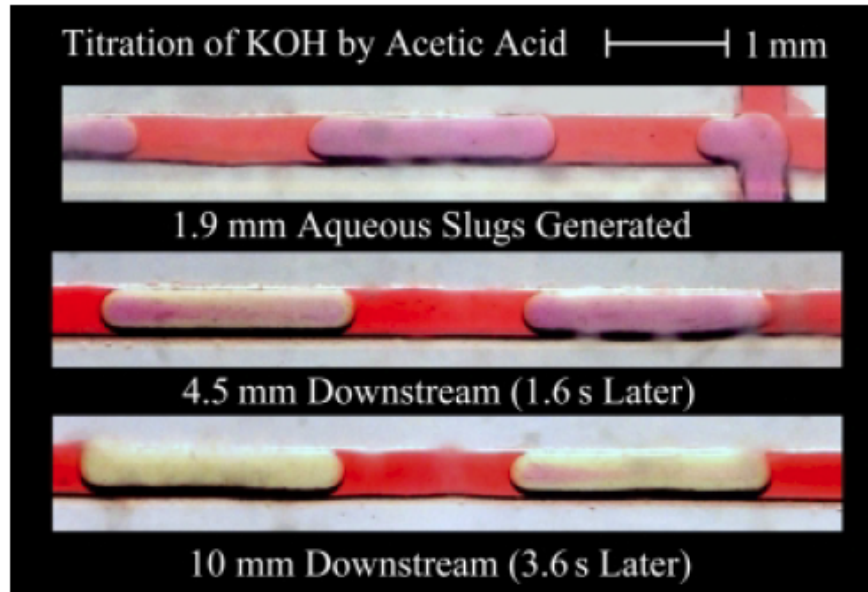


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4.

3975. It also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier-fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3976. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more

generally as therapeutic agents. *Id.* 2:20-58.

3977. It also would have been obvious to use a fluorinated carrier fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

3978. It also would have been obvious to use a fluorinated carrier fluid based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3979. Claim 31 further recites: “**a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.**”

3980. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the

perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3981. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3982. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group based on Burns (2001) in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

3983. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Burns (2001). West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

3984. Claim 31 further recites: “**and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.**”

3985. Burns (2001) satisfies this limitation. For example, Burns (2001) describes that “[v]arious methods may be used to generate slugs of liquid within a microreactor. The method

discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Burns (2001) at 10-11.

3986. Figure 4 also illustrates this process:

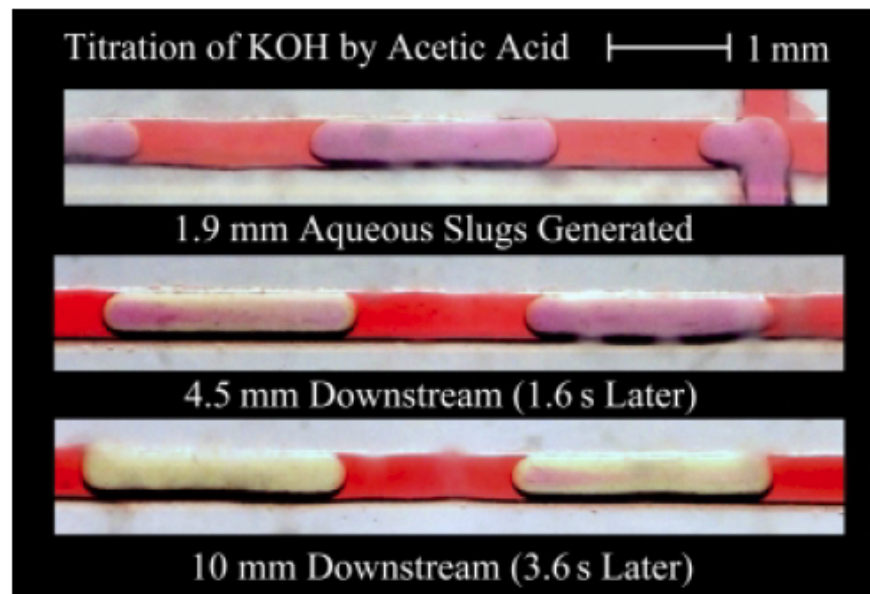


Fig. 4 Pattern of titration using acetic acid transfer into an aqueous phase containing KOH and phenol red pH indicator.

Burns (2001) at Fig. 4. Figure 4 also demonstrates that each “slug” is substantially surrounded by immiscible carrier fluid.

3987. When a less viscous fluid moves as a “plug” through a more viscous fluid in a micro-channel and when the more viscous film forms a film around the “plug”, the front of the plug becomes concave backward (towards the less viscous dispersed phase) and the back of the plug becomes concave forward towards the less viscous dispersed phase (*see* Ratulowski) to encapsulate the less viscous dispersed fluid. Such curvatures allow surface tension forces to drain the more viscous phase into and out of the film surrounding the plug. These are the curvatures

exhibited by the aqueous “slugs” in Figure 4. Based on the shape of the encapsulated fluid, these “slugs” appear to be “plugs”—i.e., the aqueous fluid was fully or substantially encapsulated by the organic phase. Based on my experience and my interpretation of Figure 4—and in particular, the shape of the “slugs” generated—it is my opinion that the “slugs” described in Burns (2001) are substantially surrounded by a thin film of oil.

3988. Claim 31 further recites: **“wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

3989. Burns (2001) at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Burns (2001) with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

3990. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

3991. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of

the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid] interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

3992. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

3993. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Burns (2001) in light of the

background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(d) Invalidity Based on Nisisako

3994. It is my opinion that Nisisako discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

3995. Claim 1 recites: “**A microfluidic system.**”

3996. Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network*.” Nisisako at Abstract (emphasis added).

3997. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in

the near future.” Nisisako at 26.

3998. Nisisako also describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *The channel for the dispersed phase is 100 μm wide and 100 μm deep*, whereas *the channel for the continuous phase is 500 μm wide and 100 μm deep*.” Nisisako at Abstract (emphasis added).

3999. The figures in Nisisako also disclose this limitation. For example, Figures 1 and 2 both show a microfluidic system:

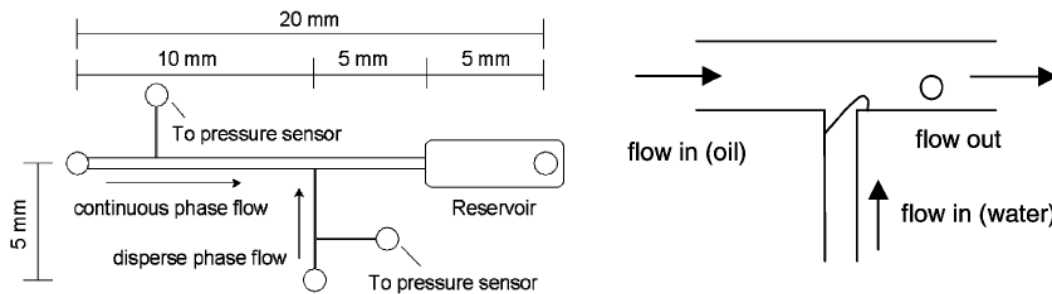


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

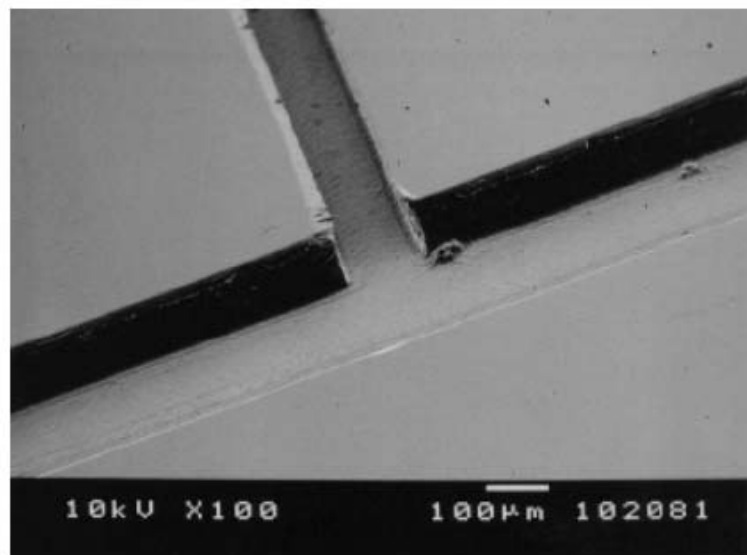


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

4000. Claim 1 further recites: “**a non-fluorinated microchannel.**”

4001. Nisisako satisfies this limitation. For example, Nisisako describes that “[a] T-junction was fabricated on a plate of *polymethyl methacrylate (PMMA)* using a 100 μ m diameter end mill. The channel surface must be hydrophobic naturally.” Nisisako at 24.

4002. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel.**”

4003. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith *oil as the continuous phase* and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

4004. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97.

Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4005. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4006. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4007. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4008. A POSA would further understand that surfactants with a hydrophobic tail and a

hydrophilic head were most commonly used with oil-water systems, such as the system described in Nisisako. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4009. Claim 1 further recites: “**at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.**”

4010. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase* and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps.”).

4011. Nisisako also discloses that the droplets are substantially encased by the carrier-fluid. For example, For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by the oil:

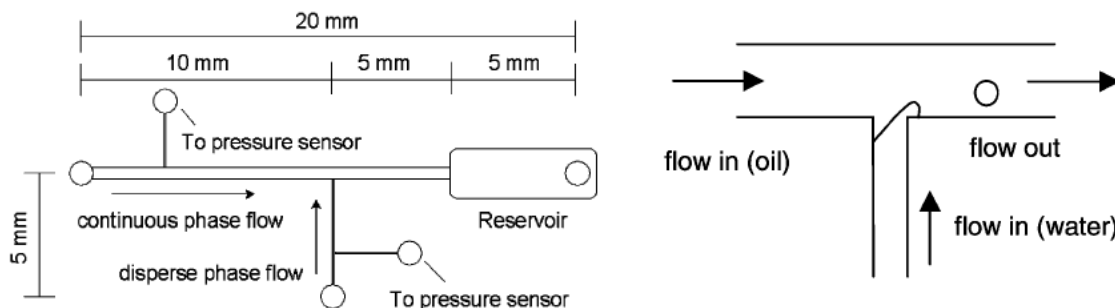


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

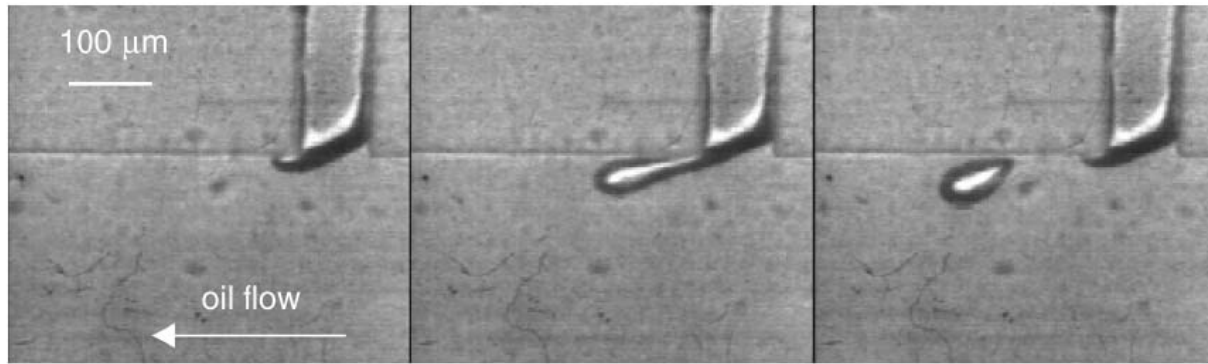


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

4012. Claim 1 further recites: **“wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

4013. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4014. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as

therapeutic agents. *Id.* 2:20-58.

4015. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4016. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4017. It also would have been obvious to use a fluorinated surfactant present at a

concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

4018. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

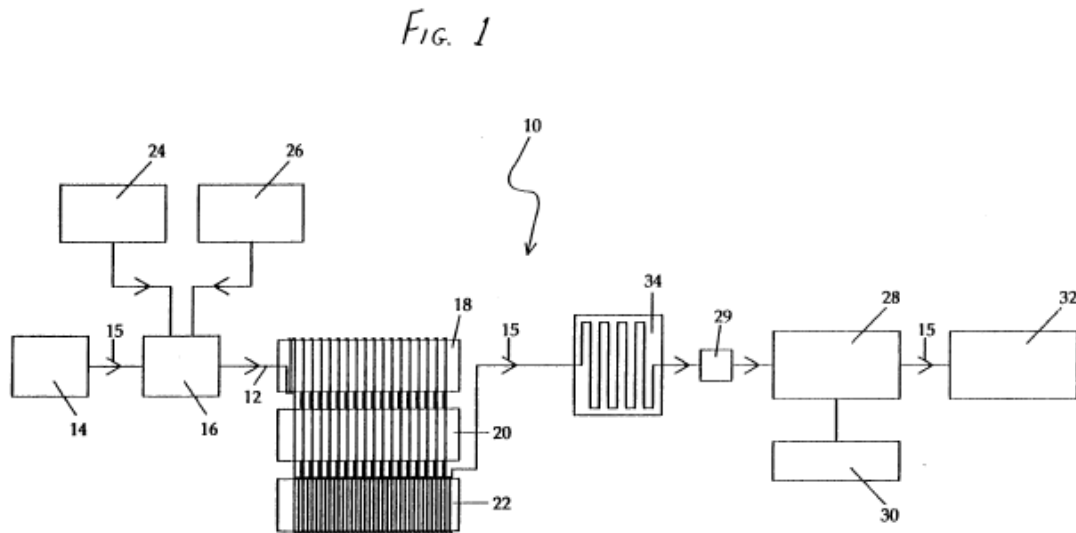
4019. Claim 2 further recites: **“the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.”**

4020. Nisisako satisfies this limitation. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

4021. While it is my opinion that Nisisako discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by

heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

4022. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally,

“[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4023. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4024. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and

Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 9*

4025. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4026. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

4027. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3585-3589, demonstrating how Quake discloses that at least one plug comprises an oligoethylene glycol.

4028. As another example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $F-(CF_2)_i-(CH_2CH_2-O)_j-H$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $FC_{7.5}E_8$) and Zonyl FSN-100 (approximately $FC_{8.2}E_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

4029. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

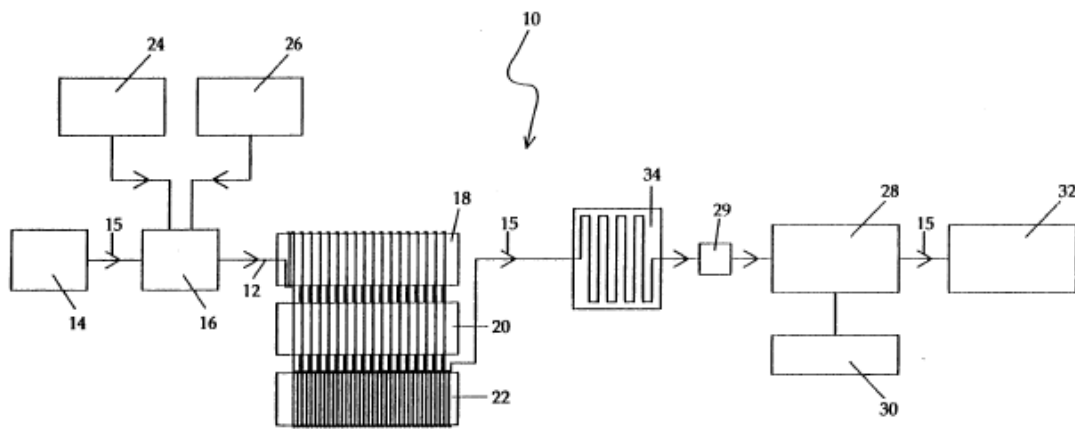
(iv) *Claim 10*

4030. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4031. Claim 10 further recites: **“the at least one plug contains at least one reagent for an autocatalytic reaction.”**

4032. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4033. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4034. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4035. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

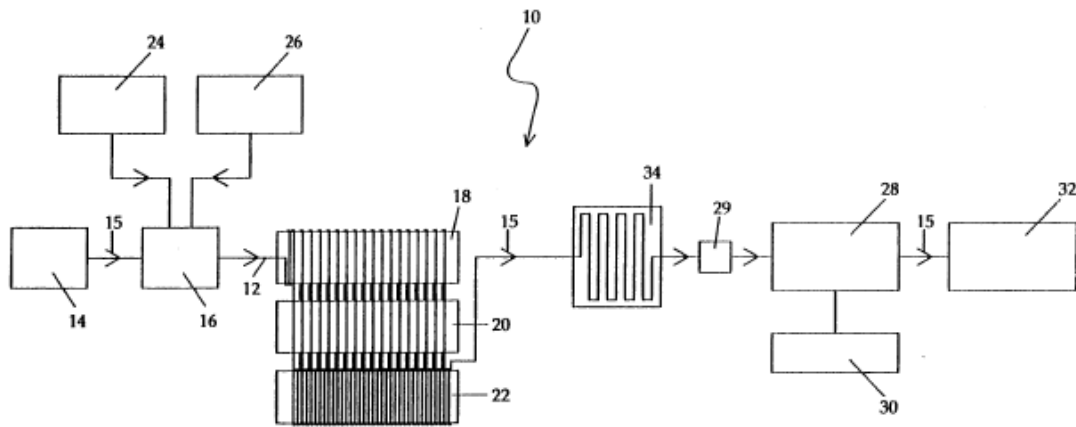
4036. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference

my analysis with respect to claims 1 and 10.

4037. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

4038. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4039. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4040. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4041. It also would have been obvious that the at least one plug contain at least one reagent for a polymerase-chain reaction based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 12*

4042. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

4043. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

4044. Nisisako satisfies this limitation. For example, Nisisako describes that “[t]he droplet size can be controlled: the minimum diameter of the droplets was about 100 μm , and the maximum 380 μm , as the flow velocity of the continuous phase was changed from 0.01 to 0.15 m s^{-1} .” Nisisako at 26. In Figure 6, Nisisako plots volume against velocity, and shows that all velocities tested, the volume of a droplet was below 100 nl:

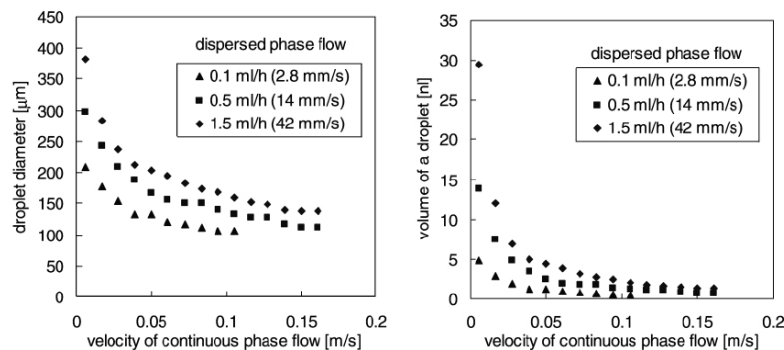


Fig. 6 Effect of velocity of continuous phase flow on droplet size (left: droplet diameter data, right: volume of a droplet calculated from the diameter).

Nisisako at Fig. 6.

(vii) *Claim 13*

4045. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4046. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

4047. Nisisako satisfies this limitation. For example, Nisisako describes that “[a] T-junction was fabricated on a plate of *polymethyl methacrylate (PMMA)* using a 100 μm diameter end mill. The channel surface must be hydrophobic naturally.” Nisisako at 24. A POSA would have understood that PMMA is a polymer.

(viii) Claim 20

4048. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

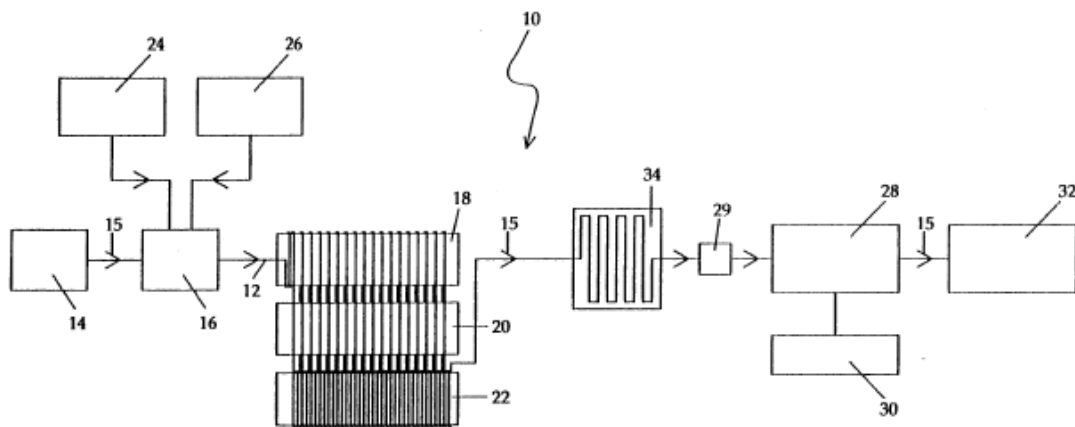
4049. I understand that the Court has not considered whether the preamble of this claim is limiting.

4050. Regardless of whether the preamble is limiting, Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[t]he past decade has seen increased interest in *chemical reactions in microfabricated devices*. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, *chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets*. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

4051. While it is my opinion that Nisisako discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62.

Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4052. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material

and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4053. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4054. It also would have been obvious to conduct a reaction within at least one plug in

view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

4055. It also would have been obvious to conduct a reaction within at least one plug based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4056. Claim 20 further recites: “**introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.**”

4057. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes carrier fluid is introduced into a microchannel of a microfluidic device. For example, Nisisako states that that “[w]ith *oil as the continuous phase* and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase

flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps.”).

4058. Figures 1 and 3 also demonstrate that the carrier fluid is introduced into a first microchannel:

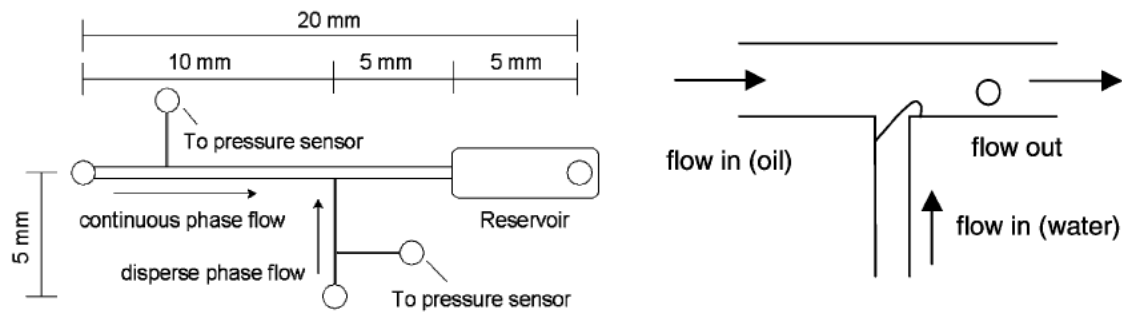


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

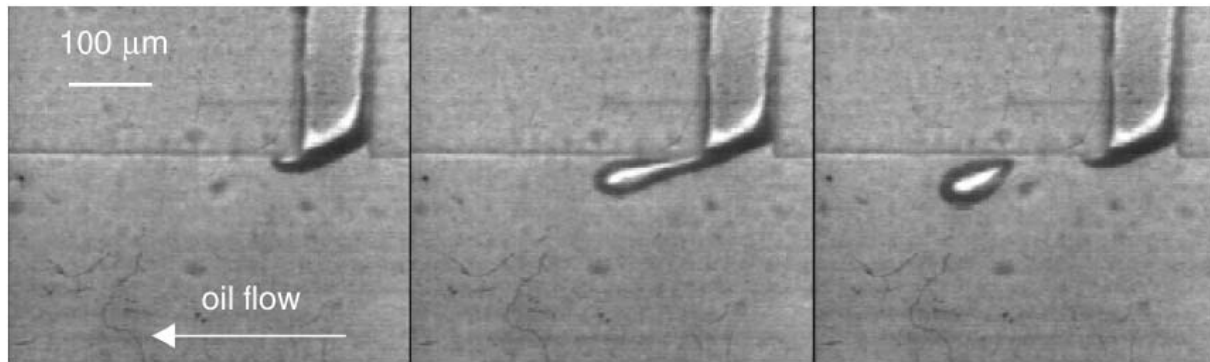


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

4059. Nisisako also discloses that a non-fluorinated microchannel. For example, Nisisako describes that “[a] T-junction was fabricated on a plate of *polymethyl methacrylate (PMMA)* using a 100 μm diameter end mill. The channel surface must be hydrophobic naturally.” Nisisako at 24.

4060. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use

a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4061. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4062. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4063. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art),

VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4064. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the system described in Nisisako. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4065. Claim 20 further recites: **“introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid.”**

4066. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.* Both are injected using syringe pumps.”).

4067. Nisisako also discloses that “[t]his method of droplet formation is shown schematically in Fig. 1.” Nisisako at 24. Figure 1 is reproduced below:

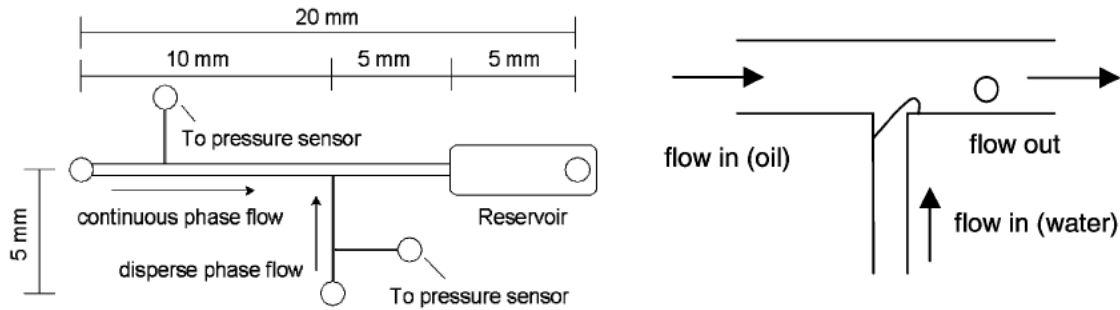


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

Nisisako at Fig. 1. Droplet formation at the T-junction is also illustrated by Figure 3:

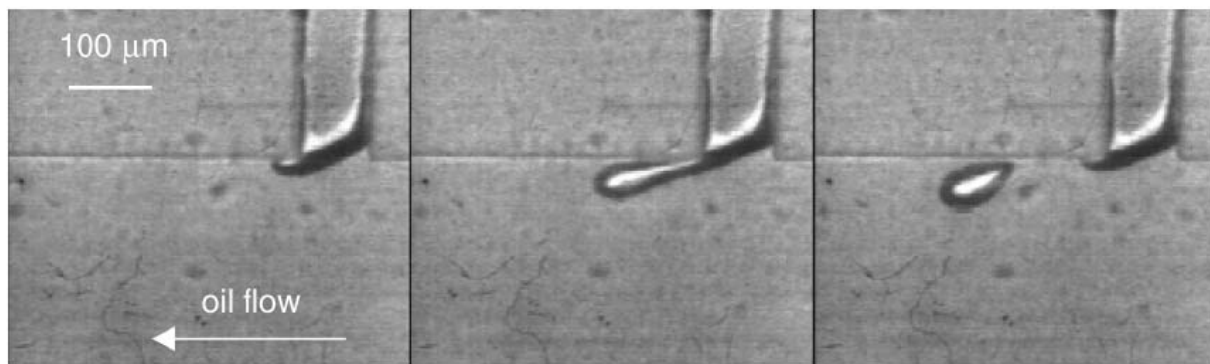


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Fig. 3; *see also* Nisisako at 25 (“Regular-sized droplets of water in oil were generated at the T-junction (Fig. 3).”

4068. Claim 20 further recites: “**wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.**”

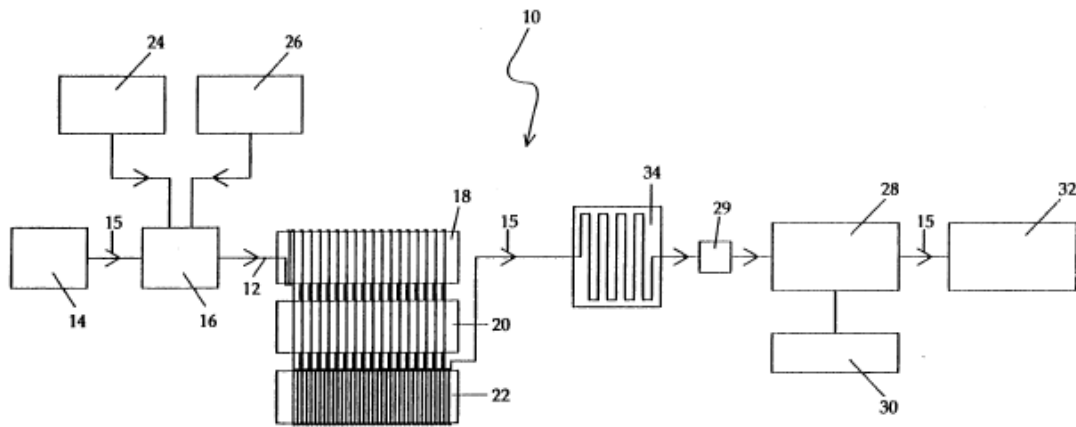
4069. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized **water droplets** can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and

continuous phase flow. *Ultra-pure water is used as the dispersed phase* and high oleic sunflower oil (triolein, 80%) as the continuous phase.”).

4070. Nisisako also makes clear that these droplets can be used for biochemical experiments and other types of reactions. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

4071. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4072. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4073. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4074. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction based on Nisiako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4075. Claim 20 further recites: “**the at least one plug-fluid is immiscible with the**

carrier-fluid.”

4076. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith *oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets* can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase and high oleic sunflower oil (triolein, 80%) as the continuous phase.*”).

4077. Claim 20 further recites: “**each plug is substantially surrounded on all sides by carrier-fluid.**”

4078. Nisisako satisfies this limitation. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added). Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by the oil:

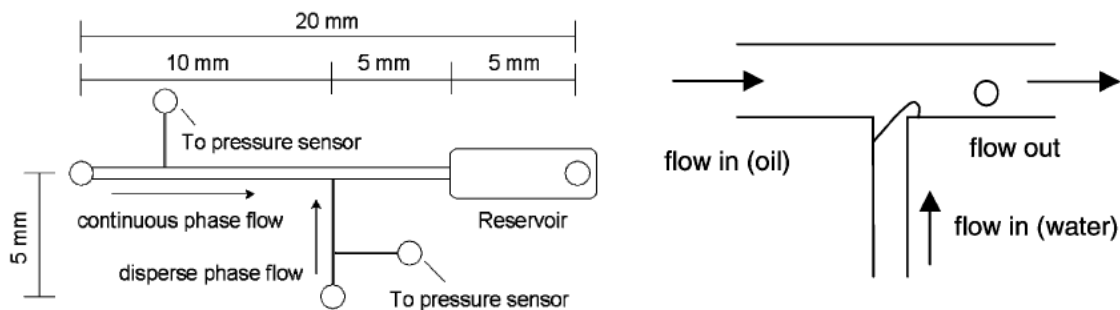


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

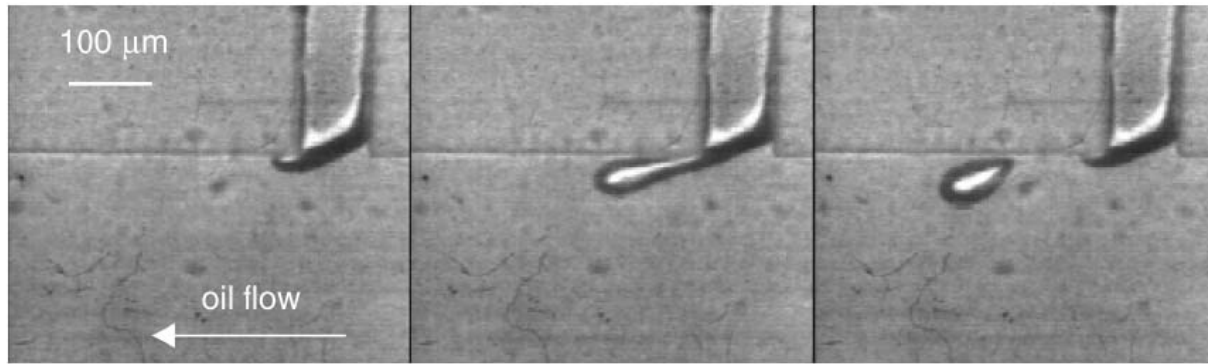


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

4079. Claim 20 further recites: **“and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

4080. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4081. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as

therapeutic agents. *Id.* 2:20-58.

4082. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4083. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4084. It also would have been obvious to use a fluorinated surfactant present at a

concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 21*

4085. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

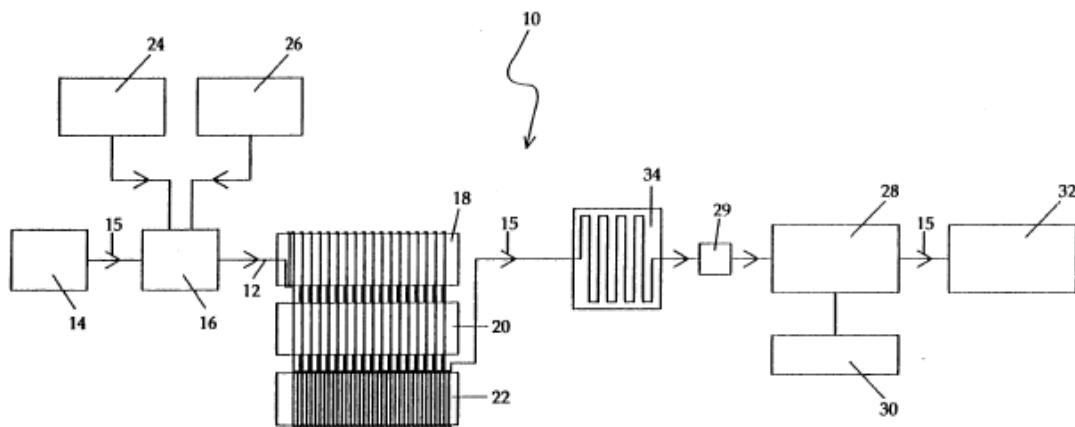
4086. Claim 21 further recites: **“the autocatalytic reaction is a polymerase-chain reaction.”**

4087. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

4088. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.”

Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4089. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material

and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4090. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4091. It also would have been obvious to conduct a polymerase-chain reaction based on

Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

4092. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

4093. Claim 22 further recites: **“the carrier-fluid comprises a fluorinated compound.”**

4094. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4095. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4096. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4097. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

4098. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

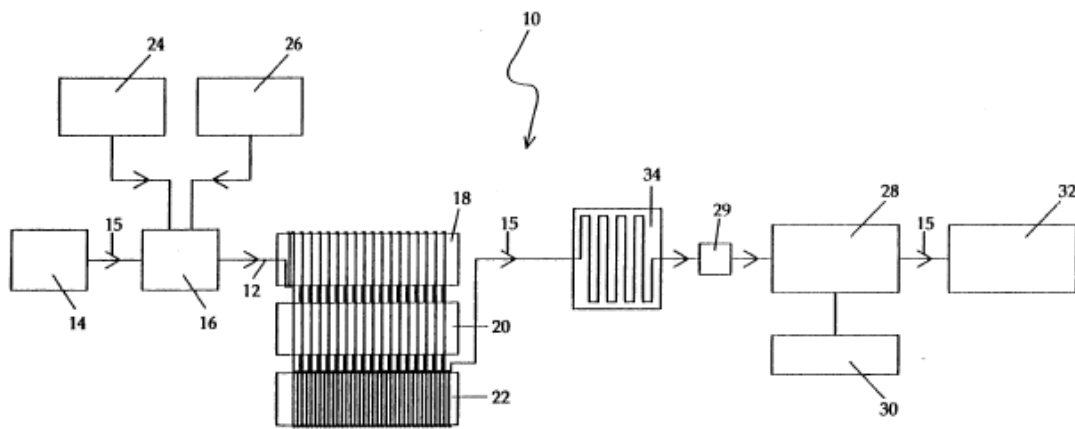
4099. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.**”

4100. Nisisako satisfies this limitation. For example, Nisisako makes clear that these droplets can be used for biochemical experiments. For example, Nisisako describes “[r]ecently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” Nisisako at 24.

4101. While it is my opinion that Nisisako discloses that at least one plug contains at

least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

4102. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4103. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4104. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 31*

4105. Claim 31 recites: “**A microfluidic system.**”

4106. Nisisako satisfies this claim limitation. For example, Nisisako discloses that “[a] method is given for *generating droplets in a microchannel network.*” Nisisako at Abstract (emphasis added).

4107. Nisisako also describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands

technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Nisisako at 24. Nisisako also describes that “[i]n order to use these generated droplets as small reaction/analysis chambers, a manipulation method is essential. In another project, we are working on electrostatic manipulation of droplets in liquid; it will be combined with this droplet preparation method in the near future.” Nisisako at 26.

4108. Nisisako also describes that “[w]ith oil as the continuous phase and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction. *The channel for the dispersed phase is 100 μm wide and 100 μm deep, whereas the channel for the continuous phase is 500 μm wide and 100 μm deep.*” Nisisako at Abstract (emphasis added).

4109. The figures in Nisisako also disclose this limitation. For example, Figures 1 and 2 both show a microfluidic system:

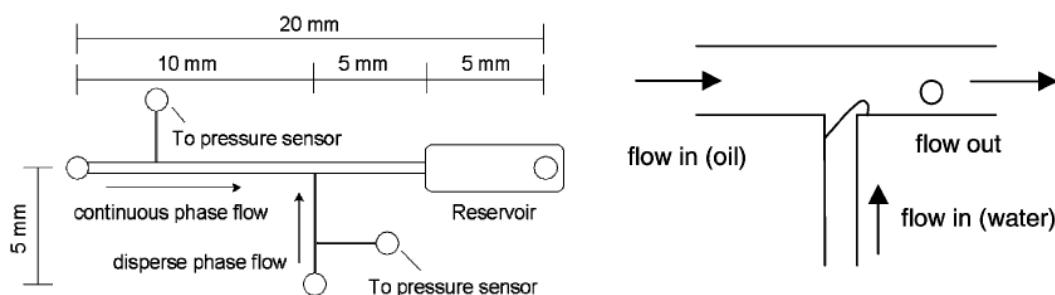


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

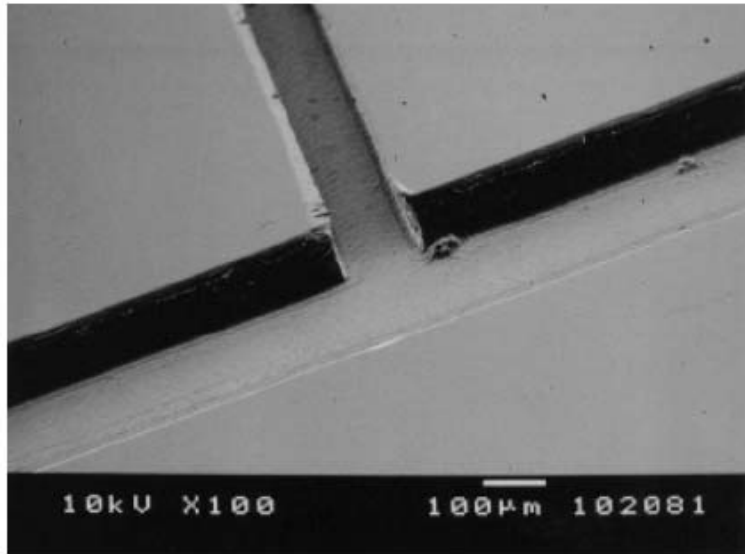


Fig. 2 SEM image of top view of the micro-channels fabricated on a PMMA plate.

Nisisako at Figs. 1 and 2.

4110. Claim 31 further recites: “**a non-fluorinated microchannel.**”

4111. Nisisako satisfies this limitation. For example, Nisisako describes that “[a] T-junction was fabricated on a plate of *polymethyl methacrylate (PMMA)* using a 100 µm diameter end mill. The channel surface must be hydrophobic naturally.” Nisisako at 24.

4112. Claim 31 further recites: “**a fluorinated carrier fluid.**”

4113. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Nisisako describes that “[w]ith *oil as the continuous phase* and water as the dispersed phase, pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. Ultra-pure water is used as the dispersed phase and *high oleic sunflower oil (triolein, 80%) as the continuous phase*. Both are injected using syringe pumps. No

surfactant is added to either phase. Semi-conductor pressure sensors (PMS-5M; Toyoda Machine Works, Japan) are set in the plate to study the conditions for droplet formation and to confirm that the flow rate is constant.”).

4114. It also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier-fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4115. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4116. It also would have been obvious to use a fluorinated carrier-fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is

required.” *Id.* at 6:46-50.

4117. It also would have been obvious to use a fluorinated carrier fluid based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4118. Claim 31 further recites: **“a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.”**

4119. Nisisako at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4120. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4121. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4122. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Nisisako. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4123. Claim 31 further recites: **“and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

4124. Nisisako satisfies this limitation. For example, Nisisako describes that “[w]ith oil as the continuous phase and water as the dispersed phase, *pico/nanoliter-sized water droplets can be generated in a continuous phase flow at a T-junction.*” Nisisako at Abstract (emphasis added); *see also* Nisisako at 25 (emphasis added) (“Syringe pumps (KDS100; Kd Scientific) are employed for injection of the dispersed phase flow and continuous phase flow. *Ultra-pure water is used as the dispersed phase* and high oleic sunflower oil (triolein, 80%) as the continuous phase. Both are injected using syringe pumps.”).

4125. Nisisako also discloses that the droplets are substantially encased by the carrier-fluid. For example, Nisisako discloses that “[a]s the *water droplets are surrounded by oil phase*, they are free from any evaporation problem.” Nisisako at 24 (emphasis added).

Figures 1 and 3 also demonstrate that the droplets are substantially surrounded by the oil:

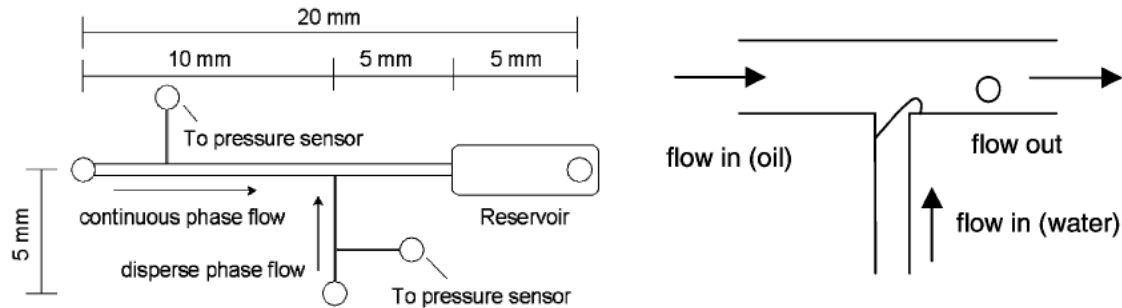


Fig. 1 Schematic of the microchannel (left) and principle of droplet formation at a T-junction (right).

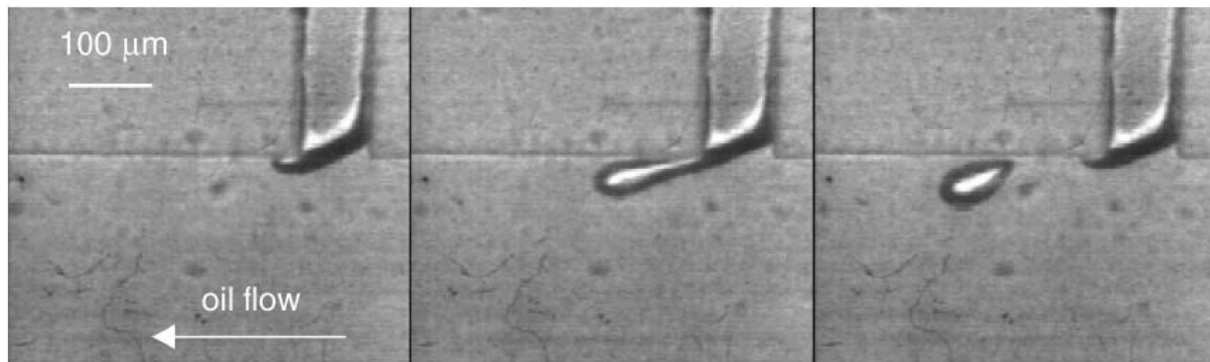


Fig. 3 Droplet formation at the T-junction (image by the high-speed video camera).

Nisisako at Figs. 1 and 3.

4126. Claim 31 further recites: “**wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

4127. Nisisako least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Nisisako with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential

for biomedical applications” and “are chemically and biologically stable.” *Id.*

4128. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4129. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4130. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the

plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4131. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Nisisako in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(e) Invalidity Based on Thorsen

4132. It is my opinion that Thorsen discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

4133. Claim 1 recites: “**A microfluidic system.**”

4134. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at the junction of two *microfluidic channels* containing water and an oil mixture, respectively.” Thorsen at 4163 (emphasis added); *see also* Thorsen at Abstract (“Here, we show that a *microfluidic device* designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

4135. Thorsen also describes that “[t]he *microfluidic devices* utilized in our experiments are fabricated by pouring acrylated urethane (Ebecryl 270, UCB Chemicals) on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x 6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. Figure 1 also shows a microfluidic system:

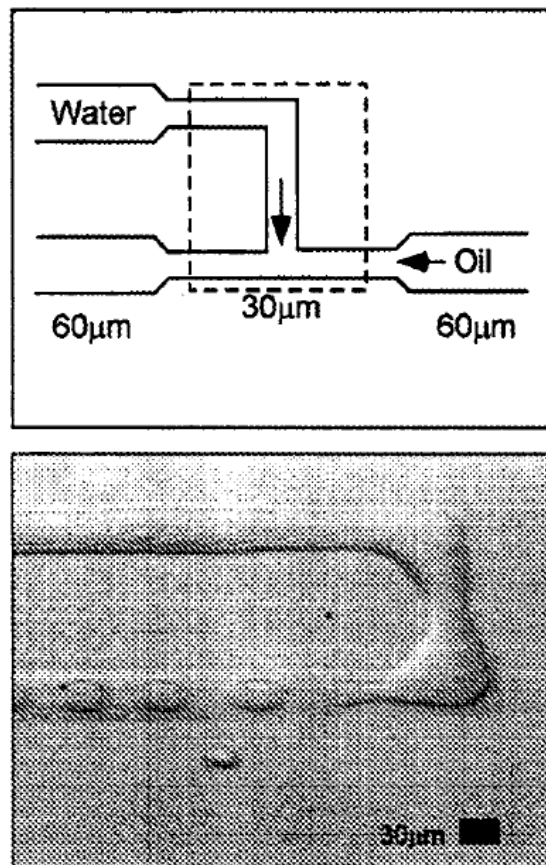


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

4136. Claim 1 further recites: “**a non-fluorinated microchannel.**”

4137. Thorsen satisfies this limitation. Thorsen describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring *acrylated urethane (Ebecryl 270, UCB Chemicals)* on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x 6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163.

4138. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel.**”

4139. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an *oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

4140. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v

/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

4141. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier-fluid and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4142. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4143. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4144. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4145. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Thorsen. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4146. Claim 1 further recites: **“at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

4147. Thorsen satisfies this limitation. For example, Thorsen states that “[t]he relative water/oil-surfactant pressures determine the size and spacing between the reverse micelles. The patterns in a rounded channel are more complex, ranging from periodic droplets to ‘ribbons,’ ‘*pearl necklaces*,’ and helical intermediate structures.” Thorsen at 4164 (emphasis added). The figures in Thorsen demonstrate that the droplets in these “pearl necklace” configurations are substantially spherical in shape:

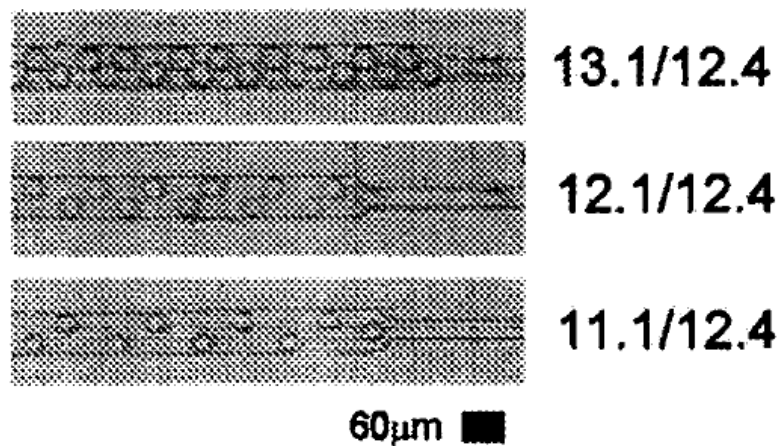


FIG. 2. Reverse micelles in square channels. Photomicrographs show the transition from the 30 μm wide channel to the 60 μm wide channel. Respective pressures for the water and oil/surfactant (hexadecane/2% Span 80) are noted in the figure.

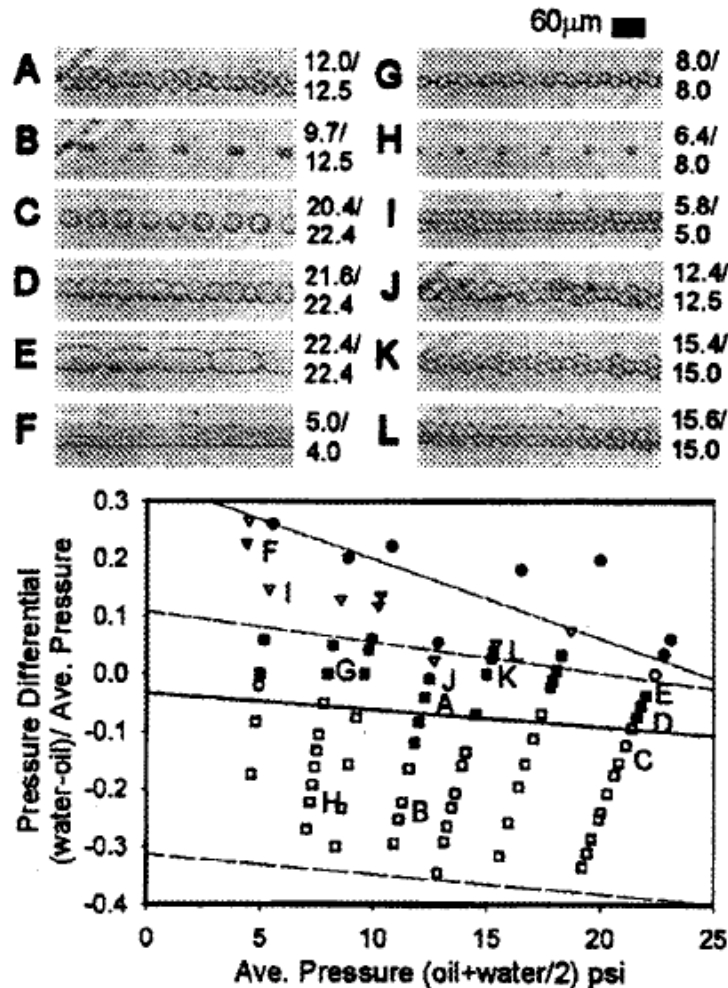


FIG. 3. Droplet patterns in rounded channels at different water and oil/surfactant pressures (noted in the figure) and the corresponding phase diagram depicting the relationship between the oil and water pressure differences and droplet morphology. Solid lines are used to define approximate boundaries between the following droplet states (top to bottom): solid water stream, ribbon layer, pearl necklace, single droplets, and solid oil stream. Symbol definition: solid water stream (solid circle); elongated droplets (open circle); triple droplet layer (solid triangle); double droplet layer (open triangle); jointed droplets (solid square); separated droplet (open square). Photomicrographs show 60 μm channel regions downstream of the point of crossflow.

Thorsen at Figs. 2 and 3.

4148. Claim 1 further recites: “wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is

higher than surface tension at the plug-fluid/carrier fluid interface.”

4149. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4150. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4151. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided

[to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4152. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4153. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

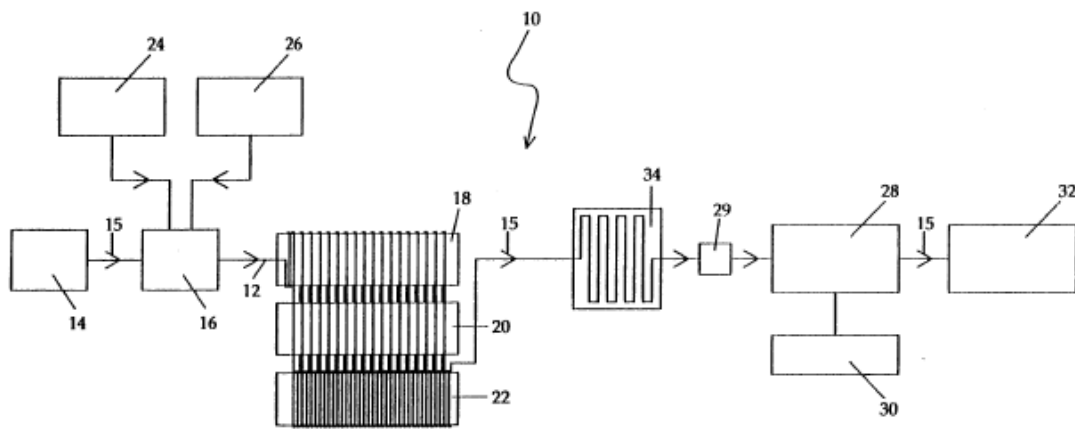
4154. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4155. Claim 2 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.**”

4156. Thorsen satisfies this limitation. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

4157. While it is my opinion that Thorsen discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

4158. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4159. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4160. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) *Claim 9*

4161. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4162. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

4163. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it

would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3585-3589, demonstrating how Quake discloses that at least one plug comprises an oligoethylene glycol.

4164. As another example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $F-(CF_2)_i-(CH_2CH_2-O)_j-H$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $FC_{7.5}E_8$) and Zonyl FSN-100 (approximately $FC_{8.2}E_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

4165. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) *Claim 10*

4166. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

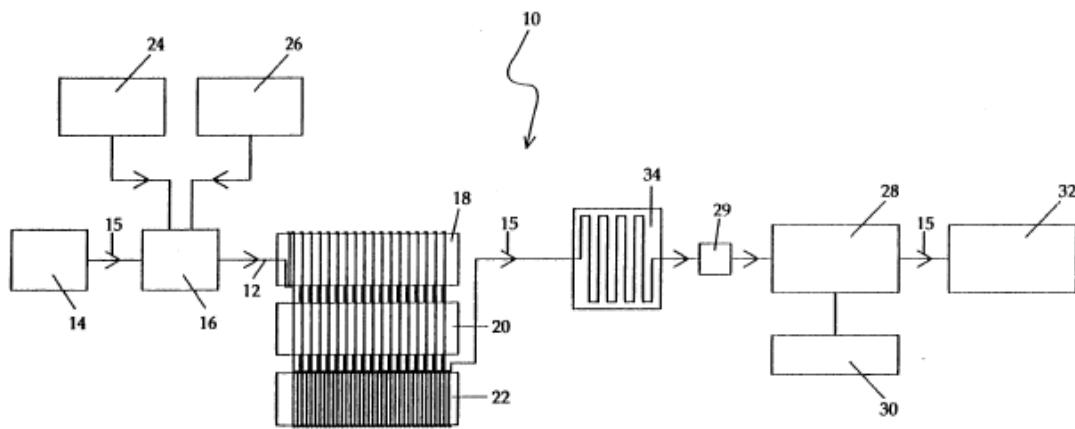
4167. Claim 10 further recites: “**the at least one plug contains at least one reagent for an autocatalytic reaction.**”

4168. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening

chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

4169. While it is my opinion that Thorsen discloses that at least one plug contains at least one reagent for an autocatalytic reaction, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4170. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4171. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4172. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

4173. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference

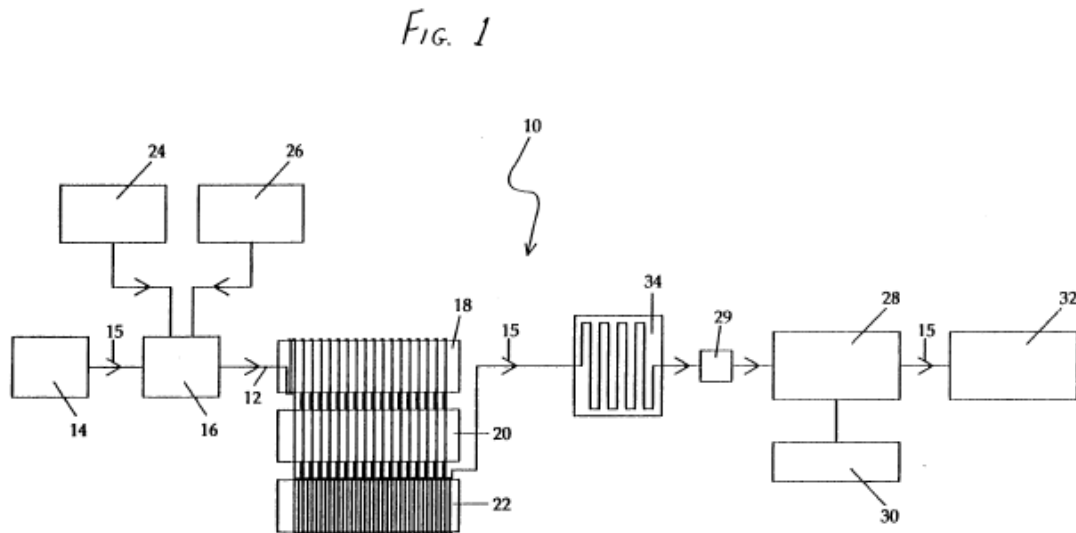
my analysis with respect to claims 1 and 10.

4174. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

4175. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung). A POSA would have recognized that screening of biological compounds could refer to DNA or RNA.

4176. While it is my opinion that Thorsen discloses a polymerase-chain reaction, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or

an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μl , is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4177. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4178. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4179. It also would have been obvious that the at least one plug contain at least one reagent for a polymerase-chain reaction based on Thorsen in light of the background knowledge

of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 12*

4180. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4181. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

4182. Thorsen satisfies this limitation. For example, Thorsen describes that “[d]roplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating *picoliter-scale* droplets.” Thorsen at 4163 (emphasis added).

(vii) *Claim 13*

4183. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4184. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

4185. Thorsen satisfies this limitation. Thorsen describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring *acrylated urethane (Ebecryl 270, UCB Chemicals)* on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x

6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. A POSA would have known that acrylated urethane is a polymer.

(viii) *Claim 20*

4186. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

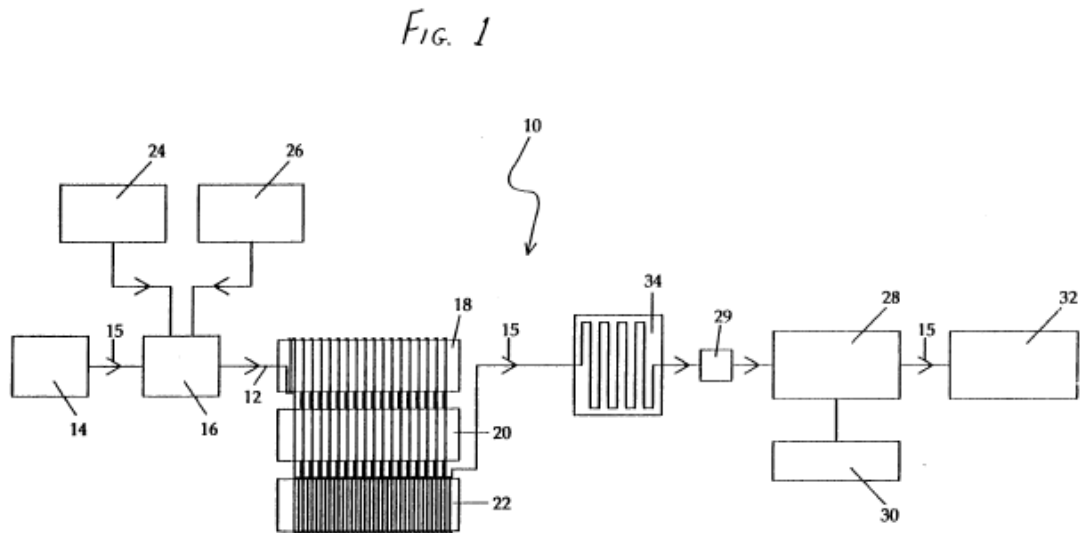
4187. I understand that the Court has not considered whether the preamble of this claim is limiting.

4188. Regardless of whether the preamble is limiting, Thorsen satisfies this claim limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively.” Thorsen at 4163.

4189. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

4190. While it is my opinion that Thorsen discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction

mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4191. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at

very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4192. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4193. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA would have recognized that a reaction could be conducted within droplets in a microfluidic system.

4194. It also would have been obvious to conduct a reaction within at least one plug based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4195. Claim 20 further recites: “**introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.**”

4196. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an *oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the

leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

4197. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

4198. Thorsen also discloses that a non-fluorinated microchannel. Thorsen describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring *acrylated urethane (Ebecryl 270, UCB Chemicals)* on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x 6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163.

4199. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier-fluid and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4200. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a

fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4201. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4202. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4203. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the system described in Thorsen. West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4204. Claim 20 further recites: **“introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid.”**

4205. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

4206. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

4207. Claim 20 further recites: **“wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.”**

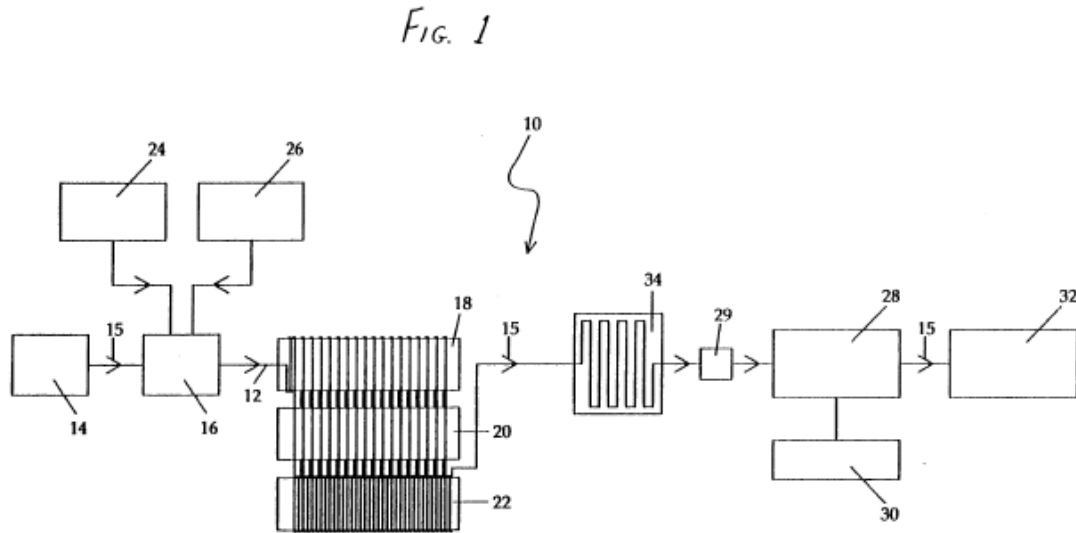
4208. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

4209. Thorsen also discloses that “[t]he fluids are introduced into the urethane microfluidic devices through pressurized reservoirs containing water and oil.” Thorsen at 4164. “As the relative water pressure is increased at fixed oil pressure, the droplets become ordered into a single continuous stream.” Thorsen at 4163.

4210. Thorsen further describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

4211. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at

8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4212. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying

vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4213. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4214. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4215. Claim 20 further recites: **“the at least one plug-fluid is immiscible with the carrier-fluid.”**

4216. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163.

4217. Claim 20 further recites: **“each plug is substantially surrounded on all sides by carrier-fluid.”**

4218. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163; Thorsen at Abstract (“Here we show that a microfluidic device designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

4219. Figure 1, showing droplets surrounded by the oil, is reproduced below:

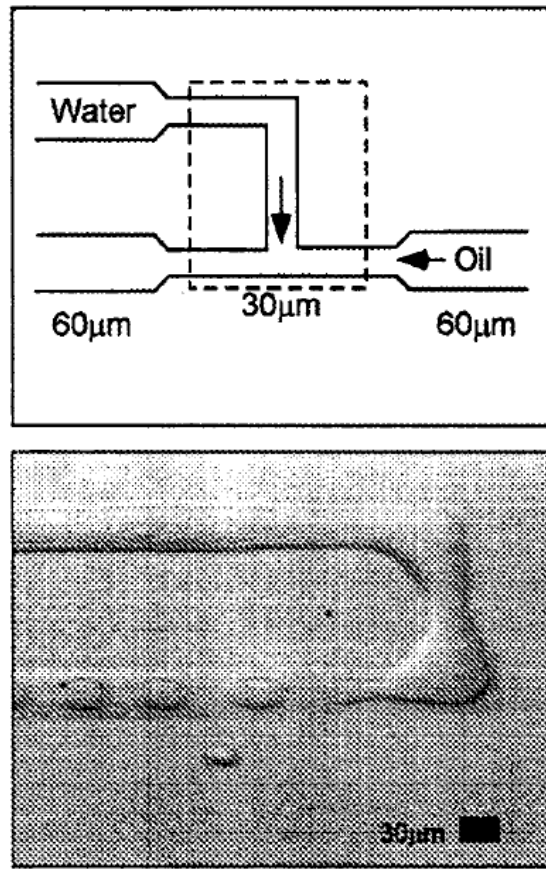


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

4220. Claim 20 further recites: “**and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

4221. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions

containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4222. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4223. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4224. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous

drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4225. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 21*

4226. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

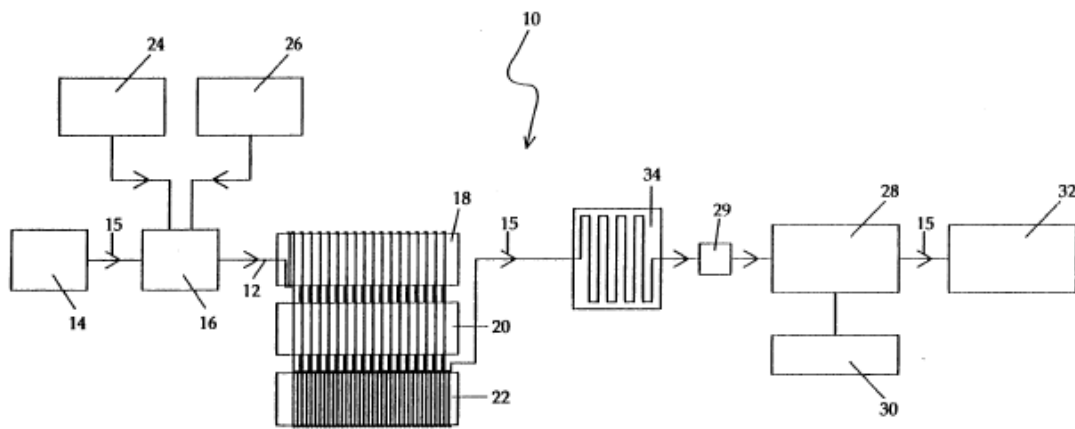
4227. Claim 21 further recites: **“the autocatalytic reaction is a polymerase-chain reaction.”**

4228. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

A POSA would have recognized that screening of biological compounds could refer to DNA or RNA.

4229. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4230. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4231. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4232. It also would have been obvious to conduct a polymerase-chain reaction based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

4233. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

4234. Claim 22 further recites: “**the carrier-fluid comprises a fluorinated compound.**”

4235. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4236. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4237. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because

they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4238. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

4239. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

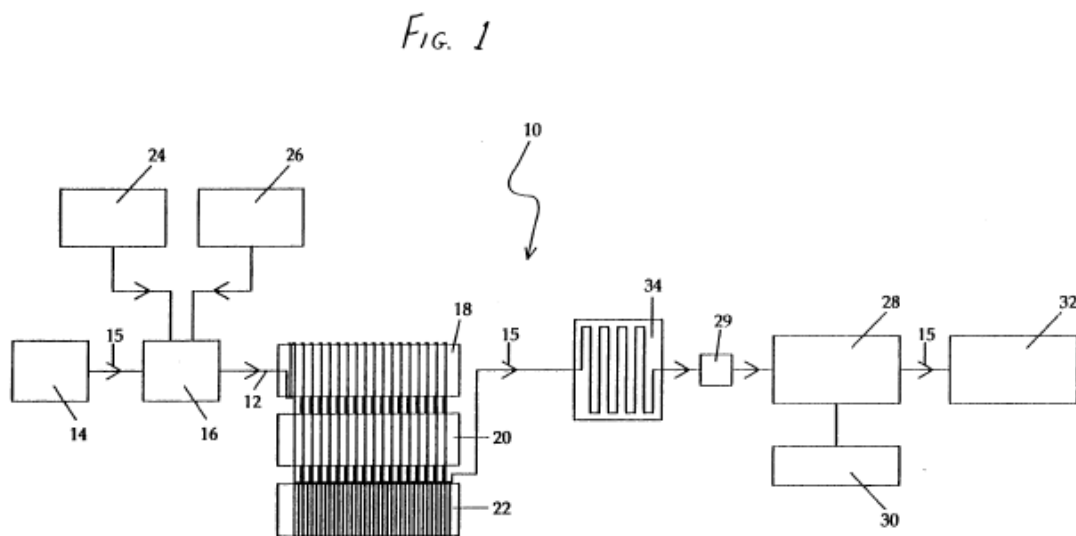
4240. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.**”

4241. Thorsen satisfies this limitation. For example, Thorsen describes that the microfluidic system described can be used for biological screening, stating that “[t]his system may also find application as a component in a microfluidic screening chip, since it has been shown that subnanoliter vesicles have significant potential as tools for screening of biological and synthetic compounds.” Thorsen at 4166 (citing Tawfik, Chiu, Kung).

4242. While it is my opinion that Thorsen discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is

immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1).

Figure 1 of Corbett is reproduced below:



Corbett at Fig. 1.

4243. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE)

analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4244. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4245. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xii) *Claim 31*

4246. Claim 31 recites: “**A microfluidic system.**”

4247. Thorsen satisfies this limitation. For example, Thorsen describes that “we accomplish droplet formation at the junction of two *microfluidic channels* containing water and an oil mixture, respectively.” Thorsen at 4163 (emphasis added); *see also* Thorsen at Abstract (“Here, we show that a *microfluidic device* designed to produce reverse micelles can generate complex, ordered patterns as it is continuously operated far from thermodynamic equilibrium.”).

4248. Thorsen also describes that “[t]he *microfluidic devices* utilized in our experiments are fabricated by pouring acrylated urethane (Ebecryl 270, UCB Chemicals) on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJR5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x 6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163. Figure 1 also shows a microfluidic system:

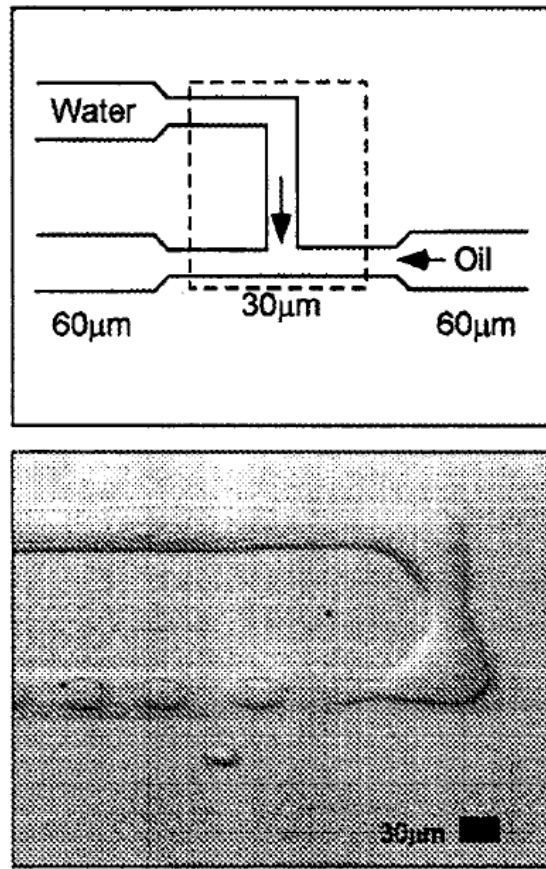


FIG. 1. Microfabricated channel dimensions at the point of crossflow and photomicrograph of the discontinuous water phase introduced into the continuous oil phase. Dashed rectangle indicates area in photomicrograph.

Thorsen at Fig. 1.

4249. Claim 31 further recites: “**a non-fluorinated microchannel.**”

4250. Thorsen satisfies this limitation. Thorsen describes that “[t]he microfluidic devices utilized in our experiments are fabricated by pouring *acrylated urethane (Ebecryl 270, UCB Chemicals)* on a silicon wafer mold containing positive-relief channels patterned in photoresist (SJ5740, Shipley), which is then cured by exposure to UV light. The channels are fully encapsulated by curing the patterned urethane on a coverslip coated with a thin layer of urethane and bonding the two layers together through an additional UV light exposure. The measured channel dimensions are approximately 60 μm wide x 9 μm high, tapering to 35 μm x

6.5 μm in the region where the water and oil/surfactant mixture meet at the crossflow intersection (Fig. 1).” Thorsen at 4163.

4251. Claim 31 further recites: “**a fluorinated carrier fluid.**”

4252. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an oil surfactant mixture, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

4253. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

4254. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier-fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4255. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or

surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4256. It also would have been obvious to use a fluorinated carrier-fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4257. It also would have been obvious to use a fluorinated carrier fluid based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4258. Claim 31 further recites: “**a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.**”

4259. Thorsen at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet

formation at the junction of two microfluidic channels containing water and an *oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the oil flow, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

4260. It also would have been obvious to combine the teachings of Thorsen with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4261. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4262. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4263. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems

described in Thorsen. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4264. Claim 31 further recites: **“and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

4265. Thorsen satisfies this limitation. For example, Thorsen states that “[t]he relative water/oil-surfactant pressures determine the size and spacing between the reverse micelles. The patterns in a rounded channel are more complex, ranging from periodic droplets to ‘ribbons,’ ‘*pearl necklaces*,’ and helical intermediate structures.” Thorsen at 4164 (emphasis added). The figures in Thorsen demonstrate that the droplets in these “pearl necklace” configurations are substantially spherical in shape:

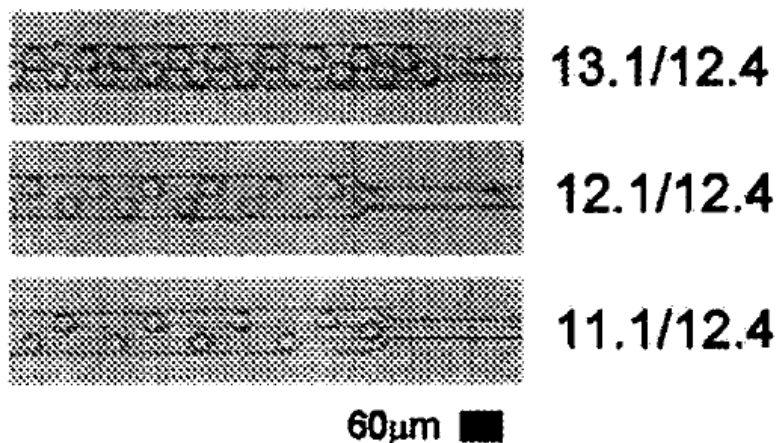


FIG. 2. Reverse micelles in square channels. Photomicrographs show the transition from the 30 μm wide channel to the 60 μm wide channel. Respective pressures for the water and oil/surfactant (hexadecane/2% Span 80) are noted in the figure.

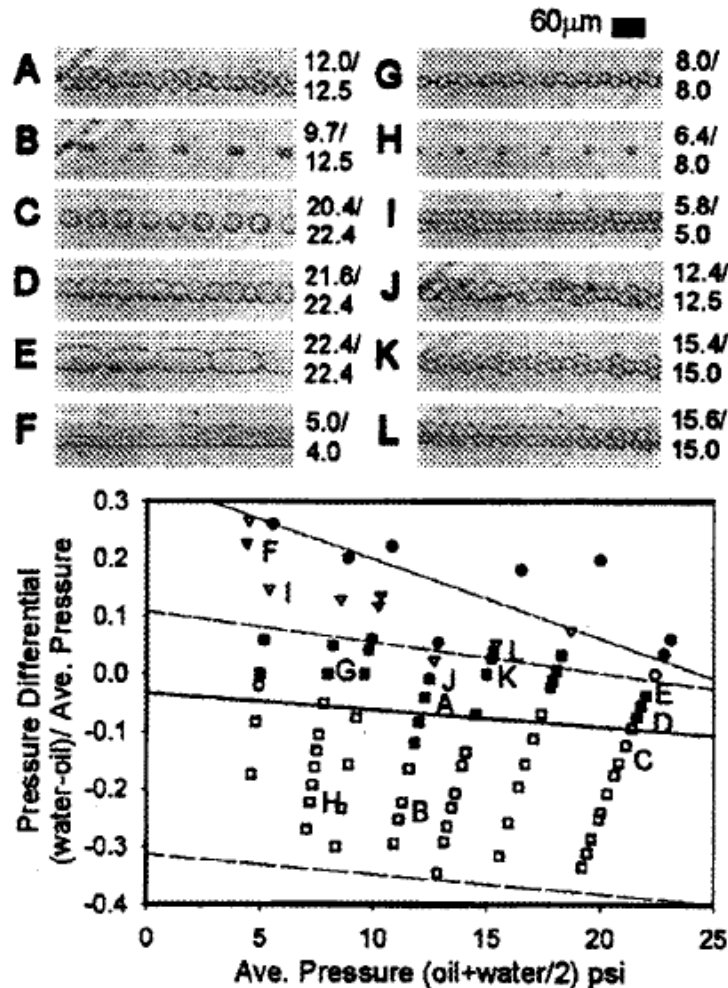


FIG. 3. Droplet patterns in rounded channels at different water and oil/surfactant pressures (noted in the figure) and the corresponding phase diagram depicting the relationship between the oil and water pressure differences and droplet morphology. Solid lines are used to define approximate boundaries between the following droplet states (top to bottom): solid water stream, ribbon layer, pearl necklace, single droplets, and solid oil stream. Symbol definition: solid water stream (solid circle); elongated droplets (open circle); triple droplet layer (solid triangle); double droplet layer (open triangle); jointed droplets (solid square); separated droplet (open square). Photomicrographs show 60 μm channel regions downstream of the point of crossflow.

Thorsen at Figs. 2 and 3

4266. Claim 31 further recites: “wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is

higher than surface tension at the plug-fluid/carrier fluid interface.”

4267. Thorsen satisfies this limitation. For example, Thorsen describes that “[e]mulsions are formed by shearing one liquid into a second immiscible one, often in the presence of a surfactant, to create small droplets . . . [W]e accomplish droplet formation at the junction of two microfluidic channels containing water and an *oil surfactant mixture*, respectively . . . Droplet formation is achieved by high shear forces generated at the leading edge of the water perpendicular to the *oil flow*, generating picoliter-scale droplets.” Thorsen at 4163 (emphasis added).

4268. Thorsen further describes that “[v]arious oils were tested in the device, including *decane, tetradecane, and hexadecane*, combined with the surfactant Span 80 concentrations (v/v) of 0.5%, 1.0%, and 2%.” Thorsen at 4164 (emphasis added).

4269. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4270. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface

tension . . . If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . . “). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4271. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4272. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Thorsen in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(f) Invalidity Based on Seki

4273. It is my opinion that Seki discloses and/or renders obvious all elements of claims 1-2, 9-13, 20-22, 26, and 31 of the '083 patent, either alone, or in light of the background

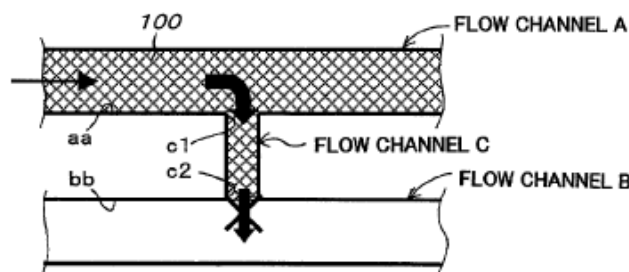
knowledge of those of ordinary skill in the art, and/or in combination with other prior art references. I understand that dependent claims include all elements of the claim(s) upon which they depend.

(i) *Claim 1*

4274. Claim 1 recites: “**A microfluidic system.**”

4275. Seki satisfies this limitation. For example, Seki describes a microfluidic system:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.



Seki at Abstract.

4276. Claim 1 further recites: “**a non-fluorinated microchannel.**”

4277. Seki discloses this limitation. For example, Seki states that “[i]n these figures, the

microchip 10 is composed of a flat plate-like base plate 12 made of a high molecular (polymeric) material such as *PDMS (polydimethyl siloxane)* and a flat plate-like surface plate 14 made of *PMMA (polymethyl methacrylate)* disposed on top 12a of the base plate 12.” Seki at [0051].

4278. Claim 1 further recites: “**a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel.**”

4279. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system with a carrier fluid in the microchannel:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4280. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97.

Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4281. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4282. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4283. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4284. A POSA would further understand that surfactants with a hydrophobic tail and a

hydrophilic head were most commonly used with oil-water systems, such as the systems described in Seki. West at 2324. Therefore, a POSA would have understood that the prior art's disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4285. Claim 1 further recites: **“at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

4286. Seki discloses this limitation. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4287. Seki also describes that the plug is substantially encased by carrier-fluid. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow

channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

4288. Claim 1 further recites: **“wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

4289. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4290. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as

therapeutic agents. *Id.* 2:20-58.

4291. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4292. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interface and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4293. It also would have been obvious to use a fluorinated surfactant present at a

concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ii) *Claim 2*

4294. Claim 2 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

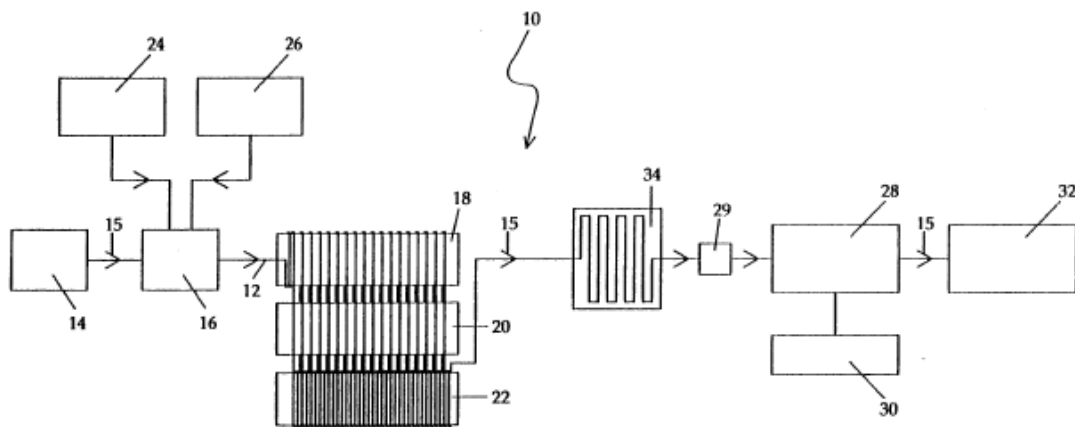
4295. Claim 2 further recites: **“the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.”**

4296. Seki discloses this limitation. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

4297. While it is my opinion that Seki discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal

cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

4298. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the

entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4299. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4300. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iii) Claim 9

4301. Claim 9 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4302. Claim 9 further recites: “**wherein the fluorinated surfactant comprises an oligoethylene glycol.**”

4303. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3585-3589, demonstrating how Quake discloses that at least one plug comprises an oligoethylene glycol.

4304. As another example, Schubert discloses a fluorinated surfactant comprising an oligoethylene glycol. Schubert describes that “[t]he fluorinated surfactants used are commercial blends of non-ionic n-alkyl polyglycol ethers with a perfluorinated alkyl chain of the type $F-(CF_2)_i-(CH_2CH_2-O)_j-H$ (denoted FC_iE_j from DuPont (Zonyl FSO-100 (approximately $FC_{7.5}E_8$) and Zonyl FSN-100 (approximately $FC_{8.2}E_{10}$)).” Schubert at 98. A POSA would have known that Zonyl is a fluorinated surfactant comprising an oligoethylene glycol.

4305. It also would have been obvious that the fluorinated surfactant comprises an oligoethylene glycol based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(iv) Claim 10

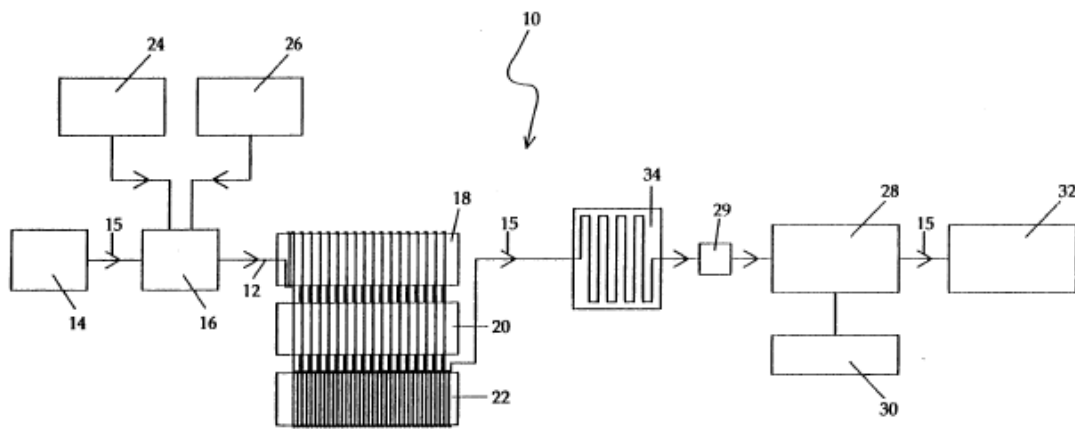
4306. Claim 10 of the '083 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

4307. Claim 10 further recites: **“the at least one plug contains at least one reagent for an autocatalytic reaction.”**

4308. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4309. It also would have been obvious that the at least one plug contains at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4310. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4311. It also would have been obvious that the at least one plug contain at least one reagent for an autocatalytic reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(v) *Claim 11*

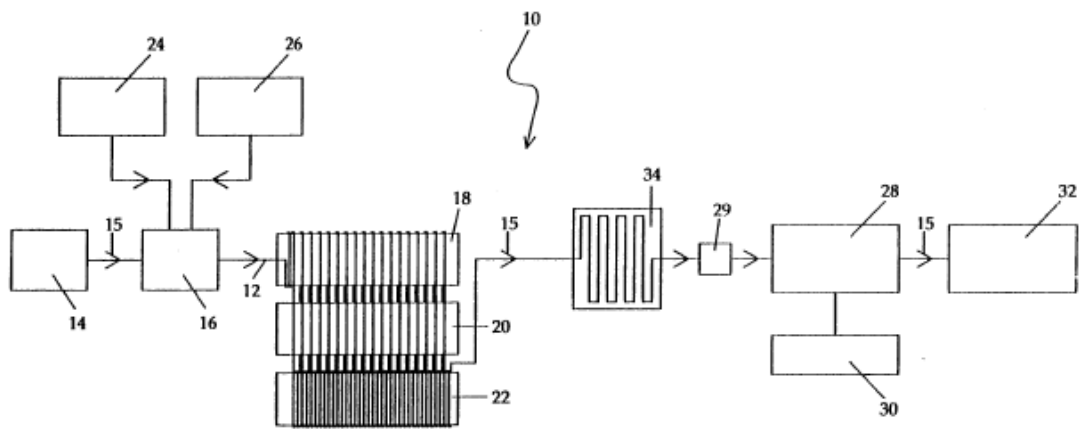
4312. Claim 11 of the '083 patent is dependent on claim 10. I incorporate by reference

my analysis with respect to claims 1 and 10.

4313. Claim 11 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

4314. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4315. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR

chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4316. It also would have been obvious to have at least one plug with a reagent for a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4317. It also would have been obvious that the at least one plug contain at least one reagent for polymerase-chain reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vi) *Claim 12*

4318. Claim 12 of the '083 patent is dependent on claim 1. I incorporate by reference

my analysis with respect to claim 1.

4319. Claim 12 further recites: “**the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.**”

4320. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki discloses that “according to the present invention, droplets having volumes corresponding to capacities of the plurality of the third flow channels can be prepared quantitatively and parallelly.” Seki at [0025]. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Quake satisfies this limitation. I incorporate my analysis with respect to ¶¶ 3604-3608, demonstrating how Quake discloses that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters.

4321. It also would have been obvious that the volume of at least one plug is between about two femtoliters and about one hundred nanoliters based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(vii) *Claim 13*

4322. Claim 13 of the '083 patent is dependent on claim 1. I incorporate by reference my analysis with respect to claim 1.

4323. Claim 13 further recites: “**the microchannel is made from a polymer, a glass, or a metal.**”

4324. Seki discloses this limitation. For example, Seki states that “[i]n these figures, the microchip 10 is composed of a flat plate-like base plate 12 made of a high molecular (polymeric)

material such as *PDMS (polydimethyl siloxane)* and a flat plate-like surface plate 14 made of *PMMA (polymethyl methacrylate)* disposed on top 12a of the base plate 12.” Seki at [0051]. A POSA would have known that both PDMS and PMMA are polymers.

(viii) *Claim 20*

4325. The preamble of claim 20 of the '083 patent recites: “**A method of conducting a reaction within at least one plug.**”

4326. I understand that the Court has not considered whether the preamble of this claim is limiting.

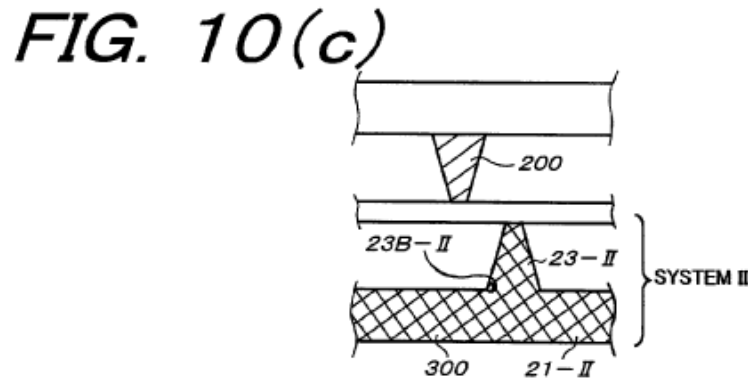
4327. Regardless of whether the preamble is limiting, Seki satisfies this claim limitation. For example, Seki describes a microfluidic system in which droplets are formed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4328. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous

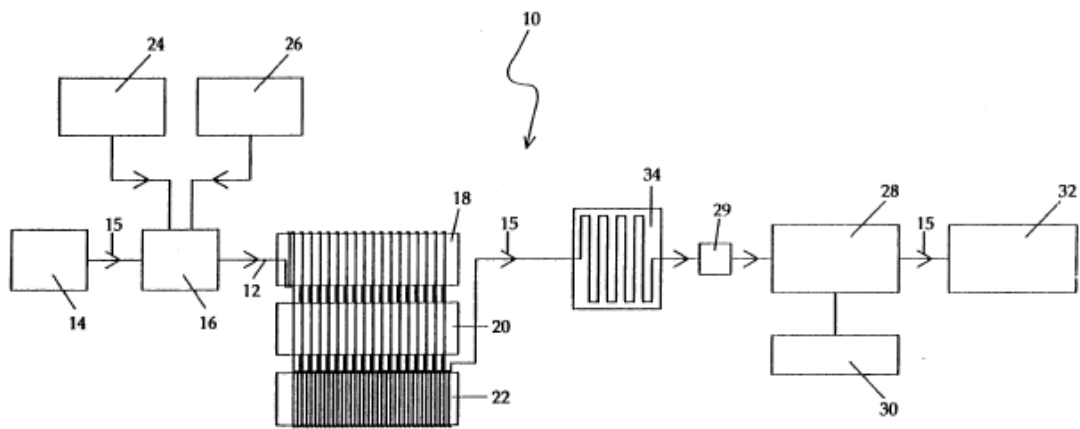
glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

4329. While it is my opinion that Seki discloses a method for conducting a reaction within at least one plug, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4330. It also would have been obvious to conduct a reaction within at least one plug in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during

amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4331. It also would have been obvious to conduct a reaction within at least one plug in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4332. It also would have been obvious to conduct a reaction within at least one plug in view of Wang. Wang describes “a method for producing small particles of micrometer or nanometer size by means of mixing reagent solution (20) and a precipitating solution (30) together in a confined reaction zone (40).” Wang at Abstract. The reagent solution and precipitating solution can be “simultaneously” injected in the carrier fluid such that they may react to form a precipitate of small particles. Wang at 2:19-26. These small particles are “micrometer or nanometer size.” Wang at 1:4-5. Based on the teachings in the art, a POSA

would have recognized that a reaction could be conducted within droplets in a microfluidic system.

4333. It also would have been obvious to conduct a reaction within at least one plug based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4334. Claim 20 further recites: **“introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device.”**

4335. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a carrier fluid being introduced into a first microchannel of a microfluidic device:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4336. Seki also discloses that the microchannel is non-fluorinated. For example, Seki states that “[i]n these figures, the microchip 10 is composed of a flat plate-like base plate 12 made of a high molecular (polymeric) material such as *PDMS (polydimethylsiloxane)* and a flat plate-like surface plate 14 made of *PMMA (polymethyl methacrylate)* disposed on top 12a of the base plate 12.” Seki at [0051].

4337. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated oil and a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4338. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4339. It also would have been obvious to use a fluorinated oil in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-

39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4340. It also would have been obvious to use a fluorinated oil and fluorinated surfactant with a hydrophilic head group based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4341. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Seki. West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4342. Claim 20 further recites: **“introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid.”**

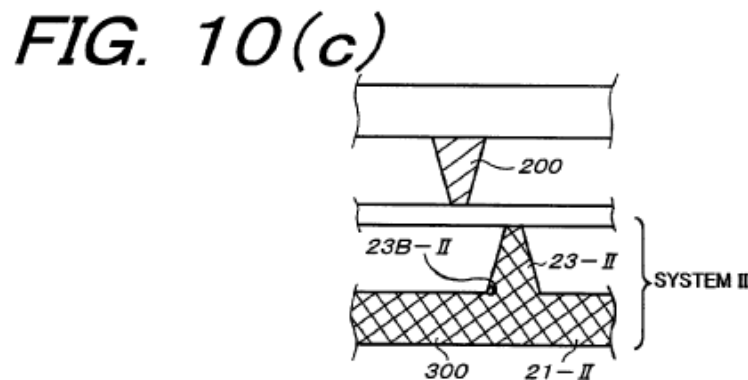
4343. Seki discloses this limitation. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that

of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4344. Seki describes that a reaction can be conducted within a droplet. “In this case, coalescence of nineteen nl of a droplet in the ten mM aqueous glucose solution 200 and nineteen nl of a droplet in the reagent 300 for analyzing glucose arises, so that then ten mM aqueous glucose solution 200 is admixed with the reagent 300 for analyzing glucose.” Seki at [0139]. The “200” and “300” numbers refer to Figure 10(c), reproduced below:



Seki at Fig. 10(c).

4345. Claim 20 further recites: “**wherein: the at least one plug fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction.**”

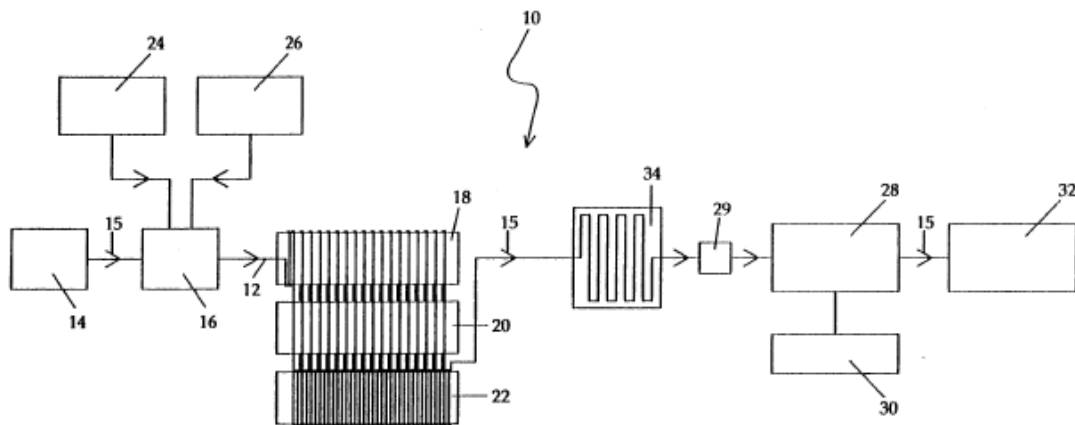
4346. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

4347. Seki also describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

4348. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the

automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4349. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at

Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4350. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA

would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4351. It also would have been obvious to use an aqueous fluid and at least one reagent for an autocatalytic reaction based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4352. Claim 20 further recites: “**the at least one plug-fluid is immiscible with the carrier-fluid.**”

4353. Seki satisfies this limitation. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

4354. Claim 20 further recites: “**each plug is substantially surrounded on all sides by carrier-fluid.**”

4355. Seki satisfies this limitation. For example, Seki describes that “a control

mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

4356. Claim 20 further recites: **“and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.”**

4357. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4358. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract.

Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4359. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4360. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic

channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4361. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(ix) *Claim 21*

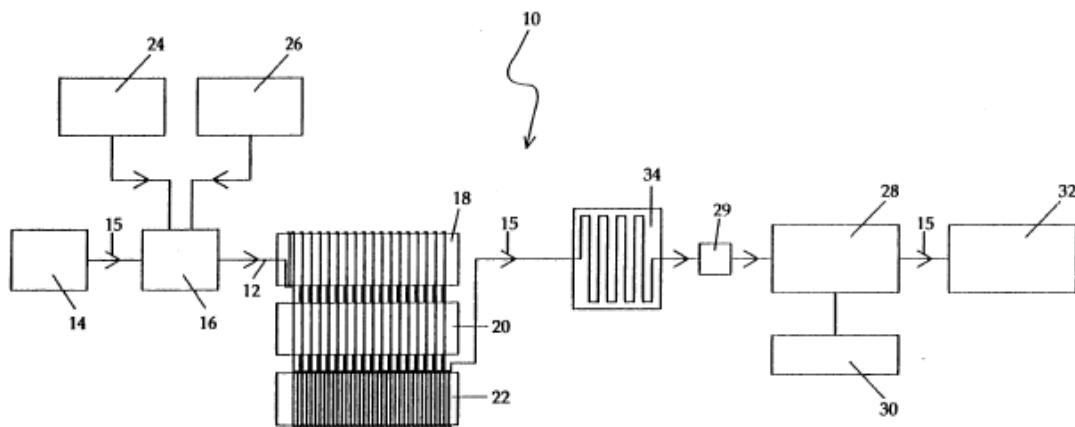
4362. Claim 21 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

4363. Claim 21 further recites: “**the autocatalytic reaction is a polymerase-chain reaction.**”

4364. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett

also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4365. It also would have been obvious to conduct a polymerase-chain reaction in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material

and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4366. It also would have been obvious to conduct a polymerase-chain reaction in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556. Based on the teachings in the art, a POSA would have recognized that PCR was one type of reaction that could be conducted within droplets in a microfluidic system.

4367. It also would have been obvious to conduct a polymerase-chain reaction based on

Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(x) *Claim 22*

4368. Claim 22 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

4369. Claim 22 further recites: **“the carrier-fluid comprises a fluorinated compound.”**

4370. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a carrier-fluid comprising a fluorinated compound with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4371. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4372. It also would have been obvious to use a carrier-fluid comprising a fluorinated

compound in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4373. It also would have been obvious to use a carrier-fluid comprising a fluorinated compound based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

(xi) *Claim 26*

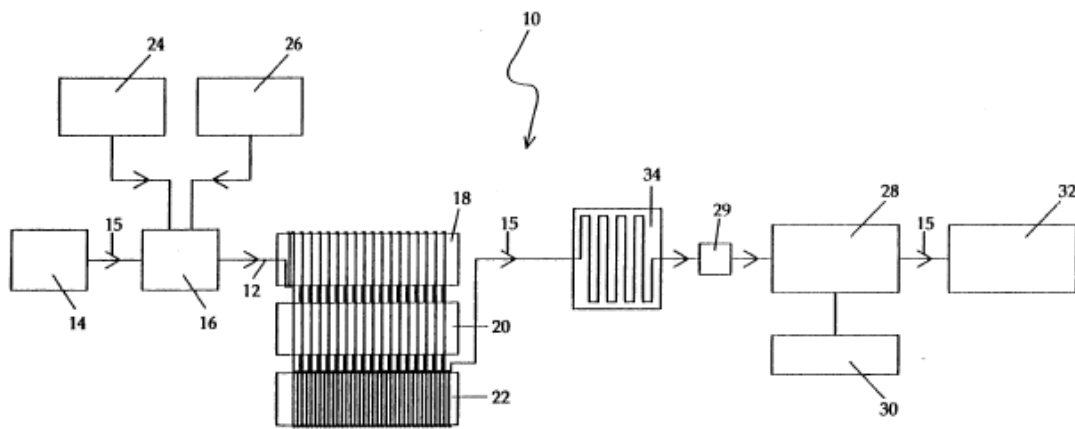
4374. Claim 26 of the '083 patent is dependent on claim 20. I incorporate by reference my analysis with respect to claim 20.

4375. Claim 26 further recites: “**the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.**”

4376. Seki discloses this limitation. Seki describes droplets comprising at least one biological molecule and at least one reagent for conducting the reaction with the at least biological molecule. For example, Seki describes that “when blood is used as a sample, it is possible to prepare a plurality of droplets from the sample blood, and a plurality of chemical reactions may be conducted in one microchip. Therefore, the operations are efficient, besides the microchip is disposable so that it is hygienic.” Seki at [0145].

4377. While it is my opinion that Seki discloses that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, Corbett describes “an apparatus and method for the amplification of a particular sequence(s) of DNA in a sample using the [polymerase] chain reaction” by injecting “a reaction mixture into a stream of carrier fluid.” Corbett at Abstract. Because “the reaction mixture is immiscible in the carrier fluid, the reaction mixture passes along with the fluid stream as a discrete pocket or plug.” Corbett at 6:60-62. Corbett also discloses that “the present invention relates to an apparatus and method for the automated cycling situation between two or more temperatures, with application, for example, in the amplification of nucleic acid sequences by heat-stable enzymes using thermal cycling.” Corbett also describes that “[a] reaction mixture comprising the sample containing the DNA sequence(s) to be amplified, thermostable DNA polymerase (or an enzymatically active fragment thereof or an enzymatically active derivative thereof or a reverse transcriptase), oligonucleotide primers, the four deoxyribonucleotide triphosphates and other desirable components in a small volume, approximately 5-20 μ l, is injected by means 24 into tube 12 via inlet port 16.” Corbett at 8:38-46 (referring to Fig. 1). Figure 1 of Corbett is reproduced below:

FIG. 1



Corbett at Fig. 1.

4378. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Lagally. Lagally describes small-volume PCR that takes place on a microfluidic plate. For example, Lagally discloses that “[s]tochastic PCR amplification of single DNA template molecules followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Lagally at Abstract. Lagally explains that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Lagally at 565. In Lagally, “[t]he PCR-CE chips were prepared for operation by forcing the separation medium through the entire microfluidic system using a syringe. The CE separation medium consisted of 0.75% (w/v) hydroxyethyl cellulose (HEC) in 1 x Tris acetate EDTA (TAE) buffer with 1 μ M thiazole orange. The gel was then evacuated from the PCR chambers and the sample bus by applying vacuum at the valve reservoir, forming a passive barrier to the flow of reagents from the PCR chamber into the separation channel during amplification. The valve and vent manifolds were clamped to the chip, and the sample was introduced at one of the sample bus reservoirs with a

pipet. The valve was opened by applying vacuum to the appropriate port on the valve manifold, and vacuum was simultaneously applied to the corresponding hydrophobic vent. Air pressure applied at the sample bus forced the sample through the valve and into the PCR chamber. The valve was then pressure-sealed (10-15 psi) to prevent sample movement during heating.” Lagally at 567.

4379. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA in view of Burns (1996). Burns (1996) describes performing PCR using “a surface-tension-based pump able to move discrete nanoliter drops through enclosed channels using only local heating.” Burns (1996) at 5556. “[S]ample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C). Burns (1996) at 5556.

4380. It also would have been obvious that at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

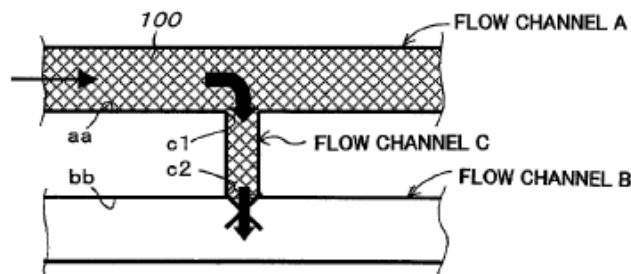
(xii) *Claim 31*

4381. Claim 31 recites: “**A microfluidic system.**”

4382. Seki satisfies this limitation. For example, Seki describes a microfluidic system:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened

on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.



Seki at Abstract.

4383. Claim 31 further recites: “**a non-fluorinated microchannel.**”

4384. Seki discloses this limitation. For example, Seki states that “[i]n these figures, the microchip 10 is composed of a flat plate-like base plate 12 made of a high molecular (polymeric) material such as *PDMS (polydimethylsiloxane)* and a flat plate-like surface plate 14 made of *PMMA (polymethyl methacrylate)* disposed on top 12a of the base plate 12.” Seki at [0051].

4385. Claim 31 further recites: “**a fluorinated carrier fluid.**”

4386. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, Seki describes a microfluidic system with a carrier fluid:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that

of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4387. It also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated carrier fluid with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4388. It also would have been obvious to use a fluorinated carrier fluid in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4389. It also would have been obvious to use a fluorinated carrier fluid in view of Ramsey. Ramsey describes “[a] method for conducting a broad range of biochemical analyses or manipulations of a series of nono- to subnano- liter reaction volumes, and an apparatus for carrying out the same.” Ramsey, at Abstract. The volumes of reaction fluid are separated by

volumes of segmenting fluid which is preferably immiscible with the reaction fluid. *Id.* at 6:36-39. Suitable segmenting fluids include: “Paraffin and mineral oils . . . because they have been used to isolate cells in small volumes of extracellular solutions without any deleterious effects. Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.” *Id.* at 6:46-50.

4390. It also would have been obvious to use a fluorinated carrier fluid based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4391. Claim 31 further recites: “**a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid.**”

4392. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with a hydrophilic head group with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.” Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4393. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated

surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4394. It also would have been obvious to use a fluorinated surfactant comprising a hydrophilic head group based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

4395. A POSA would further understand that surfactants with a hydrophobic tail and a hydrophilic head were most commonly used with oil-water systems, such as the systems described in Seki. West at 2324. Therefore, a POSA would have understood that the prior art’s disclosure of fluorinated surfactants for oil-water systems included fluorinated surfactants with hydrophilic headgroups.

4396. Claim 31 further recites: **“and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid.”**

4397. Seki discloses this limitation. For example, Seki describes a microfluidic system in which aqueous fluid is continuously flowed:

In order to be capable of handling quantitatively a liquid in a simple structure by only easy operations, a control mechanism comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the above-described third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel due to a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and

then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.

Seki at Abstract.

4398. Seki also describes that the plug is substantially encased by carrier-fluid. For example, Seki describes that “a control mechanism for a trace quantity of liquid of the present invention comprises a first flow channel and a second flow channel each extending along a predetermined direction; and a third flow channel having a thinner thickness than that of the first flow channel and that of the second flow channel; the third flow channel being opened on flow channel walls of the first flow channel and the second flow channel, respectively, whereby the third flow channel links the first flow channel to the second flow channel; a liquid introduced into the first flow channel being pulled in the third flow channel by means of a capillary phenomenon through an opening of the third flow channel opened on the flow channel wall of the first flow channel, and then, the liquid remained in the first flow channel being removed to prepare a droplet having a volume corresponding to a capacity of the third flow channel.” Seki at [0018].

4399. Claim 31 further recites: “**wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.**”

4400. Seki at least renders obvious this limitation, in light of the background knowledge of those of ordinary skill in the art and/or additional relevant prior art. For example, it also would have been obvious to combine the teachings of Seki with one or more prior art references to satisfy this element. For example, it would have been obvious to use a fluorinated surfactant with microfluidic droplets in view of Schubert. Schubert describes “[m]icroemulsions containing fluorinated oil” prepared by “mixing water, the perfluorinated oil, and a fluorinated surfactant.”

Schubert at 97. Schubert notes that “[f]luorinated compounds . . . offer the potential for biomedical applications” and “are chemically and biologically stable.” *Id.*

4401. It also would have been obvious to use a fluorinated surfactant in view of Krafft. Krafft describes emulsions comprising a fluorocarbon hydrophobic phase. Krafft, at Abstract. Exemplary fluorocarbons are provided. *Id.* at 9:24-51. A “dispersing agent” or surfactant may also be used. *Id.* at 11:5-23. Exemplary fluorinated surfactants are provided. *Id.* Krafft notes that these “fluorochemical emulsions” have been used as blood substitutes and more generally as therapeutic agents. *Id.* 2:20-58.

4402. Reducing the plug-fluid/carrier-fluid surface tension to allow for easy extrusion of the plug-fluid droplet was well known to affect the size of the plug. On the other hand, a plug-fluid/carrier-fluid surface tension that was too low could cause deformation of the plug during extrusion. For example, in 1998, Peng and Williams studied droplet detection from a single pore due to cross-flow shear force. *See* Peng at 895 (“[T]he droplet forms a sphere, which implies that the cross-flow shear force is small in comparison to the [plug-fluid/carrier-fluid]) interface tension If the contact angle . . . is too small, or the membrane surface is wetted by the dispersed phase, the droplet may adopt a [non-spherical] form . . . [that] should be avoided . . . [to produce] a well defined droplet size distribution . . .”). A POSA would have known, therefore, that the plug-fluid/carrier-fluid surface tension, should be carefully controlled through an added surfactant.

4403. At low oil flow rate regions of the microchannel, large aqueous drops may make contact with the wall, particularly if the wall is hydrophilic. At such contact points, without an encapsulating oil film, the surface tensions determine the degree of contact between the aqueous drop with the wall. In other words, surface tensions of the plug-fluid/microchannel wall interace

and the carrier-fluid/microchannel wall interface relate to the channel wall “wettability” to the plug-fluid or carrier fluid. A POSA would have understood that high surface tension at the plug-fluid/microchannel wall interface would have led to a hydrophobic channel, and that a low surface tension at plug-fluid/microchannel wall interface would have led to a hydrophilic channel. Therefore, a higher surface tension at the plug-fluid/microchannel wall interface would have been advantageous.

4404. It also would have been obvious to use a fluorinated surfactant present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface based on Seki in light of the background knowledge of those skilled in the art. *See, e.g.*, Sections VI (Principles and Background Knowledge in the Prior Art), VIII (Prior Art), and XIII.D.2 (Motivation to Combine and Reasonable Expectation of Success), and all references cited therein.

2. *Motivation to Combine and Reasonable Expectation of Success*

4405. A POSA would have seen compelling reasons to modify the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct different types of autocatalytic reactions (and in particular, PCR) in small volumes as taught by Corbett, Lagally, Burns (1996), or Wang. This is because the prior art clearly taught that reactions could be conducted within microfluidic droplets, and there were numerous advantages associated with these microfluidic droplet reactors. In particular, a POSA would have considered it obvious to modify the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct PCR based on numerous teachings in the art, including Corbett, Lagally, and Burns (1996), which discussed small-scale and even on-chip PCR (*see, e.g.*, Burns (1996)). A POSA would have had a reasonable expectation of success in so modifying, as evidenced by both the prior art and contemporaneous reports that PCR had been widely and successfully

implemented at the microfluidic scale at the time of filing. Indeed, Quake itself describes both enzymatic reactions with biological molecules and PCR within microfluidic droplets. Quake at [0080] and [0170].

4406. A POSA would have been strongly motivated to perform autocatalytic reactions, including PCR, in the microfluidic reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki because doing so would have provided the substantial benefits known to be associated with microfluidic reactors. For example, Nisisako noted that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation, and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets.” *Treating liquid samples in droplet shape has the advantage that dead volume can be decreased.* Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is *likely to become increasingly important.*” Nisisako at 24 (emphasis added).

4407. A POSA would also prefer to carry out reactions in microfluidic droplets because its small dimensions allow for reduction of diffusion time for bimolecular reactions. Biomolecular reactions require two molecules to first encounter each other by diffusion or convection-enhanced diffusion. The reaction time and reaction yield for a given reactor are then determined by the diffusion time and then the kinetic time after the molecular encounter. By reducing the diffusion time, a micro-droplet reactor can significantly enhance the reaction yield. *See, e.g.,* Burns (2001) at 10. The reduction of the diffusion time also allows for careful analyses of different kinetic times or kinetic rates, thus allowing for the selection or screening of chemical or biological catalysts. If such reactions involve thermal programming, the low thermal

capacitance of droplets also allows very rapid temperature change, thus preventing undesirable by-products.

4408. For example, with protein crystallization, the reduction of diffusion time reduces exposure to non-ideal environments during the random-walk diffusion *See, e.g.*, (Chayen). A large number of small-volume droplets can also enhance selectivity. If the concentration of the droplets is higher than the concentration of interfering agents, the concentration of the interfering agents will be lower in the droplets. *See, e.g.*, Ferrance at 200.

4409. As another example, Lagally explained that “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to *increase the speed of these assays* and to *reduce the amount of material and reagents needed*.” Lagally at 565 (emphasis added). Because PCR and other autocatalytic reactions rely on reagents that are often in limited supply—for example, sample DNA—the ability to reduce both the amount of material needed for the reaction to occur and the dead volume of the reaction would have been highly motivating. Ferrance similarly explained that “[t]he same advantages of *reduced time, sample, and reagents* brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out more efficiently, producing more product in less time with less side reactions.” Ferrance at 192 (emphasis added). The modification of Corbett, Lagally, Burns (1996), or Wang to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn would decrease operating costs. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby

increasing the speed of the overall reaction.

4410. Curcio likewise taught that “[m]initurization of the fluidic system is beneficial in two ways: it *enhances the speed of thermal equilibration of the reaction mixture*, thus allowing increased flow velocities and faster PCR. Also *analyte volumes are reduced*, thereby decreasing the consumption of polymerase and reagents, while concentrations of these components can be maintained at an optimal level.” Curcio at 7 (emphasis added).

4411. Vogelstein additionally taught that microfluidic PCR enabled a sample to be diluted into thousands of discrete reaction volumes that each contained either one template PCR molecule or no DNA molecules. Vogelstein at 9236, 9239. A POSA would have found this advantageous because individual-template PCR reactions would have enabled the detection of relatively rare mutations, dislocations, and allelic imbalances. Vogelstein at 9236, 9239.

4412. Reduction in size of the reaction vessel also allows for precise quantification of, for example, nucleic acids and pathogens. As a single template nucleic acid or pathogen can be placed in a droplet, detection of successful PCR amplification in a given number of droplets allows for digital quantification of, for example, the number of template nucleic acid or pathogens present. For the same reasons, other types of patterns, including *irregular* expression of nucleic acids, could also be quantified. A POSA would have expected that the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform different types of autocatalytic reactions, including PCR, that would enable these applications.

4413. Further, conducting PCR in microfluidic droplets would reduce potential contamination of the reaction, an issue that the prior art had recognized. *See, e.g.*, Corbett at 3:6-12 (“The most critical problem in applying nucleic acid amplification procedures, particularly in

repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of the assay.”).

4414. It was also well known that decreasing the scale of autocatalytic reactions, including PCR, to microfluidic levels provided the substantial advantage of making reactors portable. For example, Kopp explained that portable PCR microreactors could enable “[o]n-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Kopp at [1047]. Further, it was known that portable PCR reactors could aid physicians in the development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Kopp at [1047]. Thus, the prior art demonstrated that using the droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to perform PCR (and other autocatalytic reactions) would have advantageously allowed PCR and other autocatalytic reactions to be performed in point of care diagnostic applications.

4415. Additionally, using the microfluidic reactors for PCR reactions would have substantially increased the tolerance of PCR reactions to primer non-specificity. As of the filing date, it was well known that PCR reactions suffered from the limitation that the primers were not always specific to the sequence of interest but rather could also bind to other sequences. Cha at 526. Because PCR amplification reactions are exponential in nature, PCR would often be ineffective where these other DNA fragments outnumbered the fragments of interest. *Id.* In such circumstances, the amplification products of the former would greatly exceed the amplification of the latter. *Id.* By using multiple droplets, a POSA could reduce the chances of having an uncontaminated DNA template in a single reaction. *Id.* Further, a POSA could conduct

exponential amplification of the template without having the intended amplification product compete with unintended amplification products. *Id.*

4416. Moreover, a POSA would have expected the combination of microfluidic droplet reactors and different types of autocatalytic reactions, including PCR, to be successful. For example, in 2001, Lagally et al. provided an overview of the evolution of continuous flow PCR microreactors:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4

× 10⁵ template copies/μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Lagally at 565-566.

4417. In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct single-molecule DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

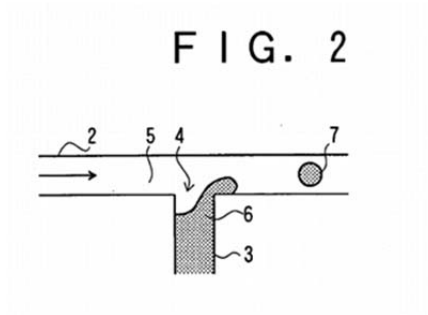
The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a

population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Lagally at 566-570. Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. Consistent with the observations in the prior art, a POSA would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform PCR reactions or other autocatalytic reactions.

4418. The fact that several other groups simultaneously developed microfluidic systems that fall within the claims of the Ismagilov patents provides further evidence that a POSA would have both found the combinations described above obvious and would have had a reasonable expectation of success in so combining. For example, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001, more than a year prior to the '083 patent's earliest claimed priority date. *See* Higuchi I-III.

4419. Higuchi I discloses "a process and apparatus for rapidly producing an emulsion and microcapsules in a simple manner." Higuchi I at Abstract. As an example, Higuchi describes that "[a] process for producing an emulsion includes a step of ejecting a dispersion phase from a dispersion phase-feeding port toward a continuous phase flowing in a microchannel in such a manner that flows of the dispersion phase and the continuous phase cross each other, whereby microdroplets are formed by the shear force of the continuous phase and the size of the microdroplets is controlled." Higuchi I at [0006]. This is illustrated by Figure 2 in Higuchi I, reproduced below:



Higuchi I at Fig. 2. In the text accompanying the figure, and corresponding with the numbers, Higuchi I describes that “[a] dispersion phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Abstract. The microfluidic droplet system Higuchi and his colleagues developed was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet systems to create droplets from continuously flowing streams of water and oil—and the use of those droplets to conduct reactions—was within the level of skill in the art as of the earliest effective priority date.

4420. As another example of, Todd Thorsen (who co-authored the Thorsen reference discussed above) also developed a droplet reactor that falls within the claims of the Ismagilov patents. Thorsen Thesis at 94-108. The Thorsen Thesis describes the following microfluidic droplet reactor:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under

monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

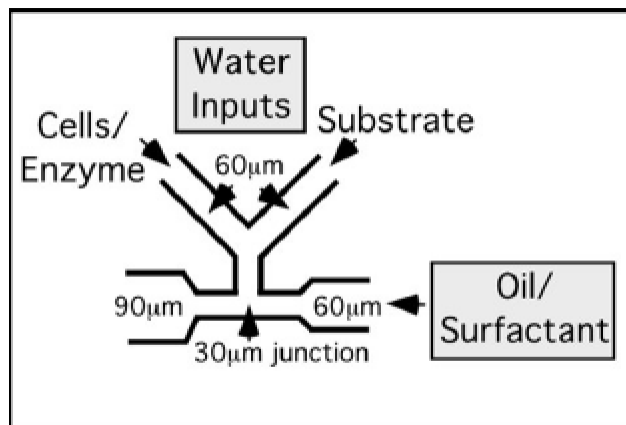


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

4421. The Thorsen thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002, suggesting that Thorsen’s work was performed before this date. The Thorsen Thesis was deposited with CalTech THESIS on December 2, 2002. Thorsen Thesis at 10X-000255686. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

4422. A POSA would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Schubert, or Krafft in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and

Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ramsey at 6:49-50 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). Krafft also noted that “the relatively low reactivity of fluorchemicals allows them to be combined with a wide variety of compounds without altering the properties of the incorporated agent.” Krafft at 2:27-30. For example, Curcio described that perfluorodecalin was utilized as a carrier fluid with small-volume PCR because “[p]erfluorocarbons are substantially more hydrophobic than hydrocarbons. Thus the interfacial surface tension between the aqueous sample and the carrier liquid will be increased, which should counteract a disintegration of the sample plugs. Additionally, the solubility of water in perfluorocarbons is extremely poor, and they show very poor affinity [and thus, high biocompatibility] towards biomolecules.” Curcio at 9. Therefore, a POSA conducting a reaction with a biological molecule in microfluidic droplets, such as PCR and other types of autocatalytic reactions, would have used fluorinated oils and fluorinated surfactants with these microfluidic droplet systems. For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

4423. Further, fluorinated oil offers high immiscibility with water and low solubility of biomolecules. *See, e.g.*, Schubert at 97 (“Fluorinated compounds also offer the potential for biomedical applications. For example, . . . fluorinated alkanes are . . . chemically and biologically stable.”); *id.* (“Because fluorocarbons are insoluble in water, however, they are currently administered in the form of emulsions, the formation of which requires the use of surfactants.”). Unlike most mineral oils, fluorinated oil has a density higher than water. Gelest at

19. This higher density allows easy separation of aqueous droplets from the oil when the emulsion is collected off the substrate.

4424. The art had also already noted that fluorination was preferable for silicon-based microfluidic devices, which have a tendency to swell when exposed to hydrocarbon oils. *See* Quake at [0118] (emphasis added) (“**TEFLON [which contains fluorination] is particularly suitable for silicon elastomer (RTV) channels**, which are hydrophobic and advantageously do not absorb water, but *they may tend to swell when exposed to an oil phase.*”). As Quake noted, “[s]welling may alter channel dimensions and shape, and may even close off channels, or may affect the integrity of the chip, for example, by stressing the seal between the elastomer and a coverslip.” Quake at [0118]. This issue was also prevalent with PDMS, a silicon material that was commonly used to manufacture microfluidic substrates. *See* Quake at [0216] (emphasis added) (“In a preferred embodiment, the invention provides a “T” or “Y” shaped series of channels molded into optically transparent silicon rubber or PolyDiMethylSiloxane (PDMS), **preferably PDMS.**”); ’407 patent at 16:59-61 (“Channels may be molded onto optically transparent silicon rubber or polydimethylsiloxane (PDMS), **preferably PDMS.**”).⁶⁸ Unlike other organic oils, fluorinated oil does not cause polymer like PDMS to swell. Holtze at 1632 (“In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.”) (citing Lee). Therefore, a POSA would have been motivated to use fluorinated oils and surfactants to prevent swelling of the polymer substrate.

4425. Importantly, fluorinated oil is far less viscous than other oils, including mineral oils. *See generally* Gelest. Instead, fluorinated oil has a viscosity similar to water. *Id.* Using a

⁶⁸ I note that this language in the Ismagilov patents was copied almost directly from Quake.

fluorinated oil with a microfluidic droplet device would thus allow high-frequency generation of droplets and parallel generation with multiple orifices. The prior art had already shown that high-throughput droplet generation was desirable. *See* Quake at [0079] (“This arrangement can be used to improve throughput or for successive sample enrichment, and can be adapted to provide a very high throughput to the microfluidic devices that exceeds the capacity permitted by conventional flow sorters.”); Quake at [0093] (“Monodisperse droplets may be particularly preferabl[e], e.g., in high throughput devices and other embodiments where it is desirable to generate droplets at high frequency.”). Further, the viscosity of fluorinated oil is insensitive to temperature, which is particular useful for DNA amplification reactions involving temperature changes. This of course includes PCR. Mullis at 9:55-60. For these reasons, a POSA would have been motivated to use fluorinated oil to achieve higher frequency droplet generation. Indeed, fluorinated oil has become the preferred carrier fluid for high-throughput aqueous droplet microfluidics. Autour at Section 4.1.

4426. As the prior art demonstrates, a POSA would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Burns (2001), Nisisako, Thorsen, or Seki to conduct autocatalytic reactions, including PCR, as taught by Corbett, Lagally, Burns (1996), or Wang.

XIV. SECONDARY CONSIDERATIONS

4427. I have also considered so-called “secondary indicia” or “secondary considerations” that may be used to demonstrate non-obviousness, and have concluded that none of these come close to overcoming the strong showing of obviousness based on multiple grounds and references, as set forth above. Following is an evaluation of these secondary indicia, with a focus on those factors that Bio-Rad identifies as purportedly supporting its non-obviousness arguments in this case. *See* Plaintiffs’ Response to Interrogatory No. 16. Should Bio-Rad be

permitted to present additional evidence or contentions regarding secondary consideration (and I understand that 10X's position is that it should not be permitted), I reserve the right to present additional responsive analysis and opinions.

A. Praise of Others

4428. I understand that Bio-Rad is contending that “[t]he extensive praise Dr. Ismagilov and his colleagues have received for pioneering general purpose techniques for conducting chemical reactions in droplets demonstrates the non-obviousness of the claimed inventions.” Plaintiffs’ Response to Interrogatory No. 16. Specifically, Bio-Rad has asserted “praise” in the form of citations to publications from Dr. Ismagilov’s group, descriptions of Dr. Ismagilov’s work in scientific articles, and awards received by various RainDance products. *Id.*

4429. Bio-Rad asserts that “in total, Dr. Ismagilov’s publications regarding his work on droplet-based microfluidics and performing reactions in such systems have been cited over 4,000 times” and because of “his high citation impact scores, Thomson Reuters named Dr. Ismagilov one of the world’s top 100 most influential chemists during the period from 2000-2011.” Plaintiffs’ Response to Interrogatory No. 16. I understand that in a related proceeding before the Patent Office, Bio-Rad has relied on citations to and descriptions Song, H., et al., “A Microfluidic System for Controlling Reaction Networks in Time,” *Angew. Chem. Int. Ed.*, 42:767-772 (2003) (“Song”) (10X-000255650-55), to support this argument. Bio-Rad asserts that this article has “been cited nearly 450 times.” *See, e.g.*, IPR2015-01156 POPR at 51. But citation numbers in excess of 400 is not unusual. For example, Thorsen has been cited nearly 1,100 times. Web of Science Citation (10X-000255953-6221).

4430. Further, with respect to specific statements that Bio-Rad asserts indicate praise, Bio-Rad falls far short of satisfying what I understand is the legal requirement to show a “nexus” between this alleged “praise” and the actual features of the Ismagilov patents.

4431. For example, Bio-Rad relies on the following statement from Fidalgo, L., et al., “Surface-induced droplet fusion in microfluidic devices,” *Lab Chip*, 7:984-986 (2007) (“Fidalgo”) (10X-000255231-42):

Microdroplets formed within microfluidic devices present a unique platform for the miniaturization of chemical and biochemical reactions.¹ The principal benefits lie in the ability to create nanolitre to femtoliter-sized individual reactors in which the contents can be accurately controlled.^{2,3} This is of great interest for areas of research where a multitude of reaction conditions with minimum amounts of reactants need to be tested, e.g., in high-throughput screening of chemical reactions, protein crystallization, and enzyme kinetics.^{4,5}

4432. Bio-Rad asserted that Fidalgo et al. “cited [Ismagilov’s] work for its teaching of a ‘unique platform’ for miniaturizing chemical reactions.” Fidalgo et al. citation “1” is to Song, H., et al., “Reactions in Droplets in Microfluidic Channels,” *Angew. Chem. Int. Ed.*, 45:7336-7356 (2006) (“Song Review”) (RI00139536-56). The Song Review is a review article meaning that it sets forth various works, including works by others, on the topic of “Reactions in Droplets in Microfluidic Channels.” For example, the Song Review discusses Thorsen (citation [35]), Nisisako (citation [36]), and Burns (2001) (citation [187]), among over two hundred other references. Accordingly, this citation would not be viewed by a POSA as praise for the work of Ismagilov’s group specifically.

4433. As another example, Bio-Rad relies on the following statement from Zimmerman, W., “Microfluidics: History, Theory and Applications,” Chapter 11 (2006) (“Zimmerman”) (10X-000255867-80):

Nonetheless, there is a clear need for simple design tools for heterogeneous systems in microchannel reactors. Engineers need to be able [to/sic] schedule “microchannel unit operations” to exploit the promise of tight control for microchannel processing, to achieve high-throughput, parallel, and dense packed production or analysis. ***Thus, batches of reagents must be deliverable by***

microchannel transport, switchable on demand. (9) in a seminal paper has shown how to generate and then model mixing and reaction within single, isolated droplets, each one of which can be directed automatically due to precise knowledge of its current position. The generation mechanism entrains vorticity in the droplet, which then serves as a chemical micromixer following the analogy with the “baker’s transformation” as a mechanistic paradigm for chaotic advection (13). Helen Song, a colleague of Rustem Ismagilov, reported on this work during the CISM workshop. The success of their engineering leads to the obvious extension – if transported droplets can be treated as pseudo-batch reactors, under what circumstances can layered flows be treated as plug-flow tubular reactors, and are there other arrangements for the reaction engineering that permit droplets to be treated as batch reactors, in the frame of reference of the droplet?

See, e.g., IPR2015-01156 POPR at 53.

4434. But this statement in Zimmerman relates to mixing in plugs. None of the asserted claims of the Ismagilov patents include limitations relating to “mixing.” Further, as discussed above, Bio-Rad has interpreted the claims of the Ismagilov patents to extend to reaction in plugs outside of the substrate. Under such conditions, any benefits relating to “mixing” and “precise knowledge of [a droplet’s] current position” are not applicable.

4435. Moreover, this allegedly inventive concept was actually well-known in the prior art. The concept of generating flow with “vorticity” in droplets to achieve high mixing was described in Burns (2001). *See, e.g.,* Burns (2001), Fig. 1. The mass transfer coefficient estimated in equation (10) in Burns (2001) demonstrates a mass transfer coefficient that is much higher than pure diffusion and is proportional to the droplet velocity. *Id.* at 13. This supports high-vorticity convection dominated mixing. “Chaotic mixing” due to nonlinear fluid particle dynamics like “baker’s transformation,” which is referenced in Zimmerman, is much weaker than convection-enhanced mixing. Kopelevich, D., and Chang, H.-C., “Nonequilibrium

Diffusion in Zeolites due to Deterministic Hamiltonian Chaos,” *Physical Rev Letter*, 83:1590 (1999) (“Kopelevich”) (10X-000255368-71); Roberts, R., and Chang, H.-C., “Wave-enhanced interfacial transfer,” *Chemical Engineering Science*, 55:1127 (2000) (“Roberts”) (10X-000255608-22). Using convection to enhance diffusion time and estimate kinetics was a well-known engineering principle that was implemented into microfluidic substrates as early as 1998. For example, Knight et al explained “many liquid phase chemical and biological processes exhibit dynamics that cannot be resolved in reaction kinetics experiments because they are faster than the mix time of conventional mixers.” Knight, B., et al., “Hydrodynamic Focusing on a Silicon Chip: Mixing Nanoliters in Microseconds,” *Physical Review Letters*, 80:3863-3866 (1998) (“Knight”) (10X-000255364-67) at 3863. To overcome this issue, Knight used flow focusing to produce a “continuous flow mixer capable of mix time of less than 10 μ s.” *Id.* at Abstract. Convection enhanced diffusion with two parallel flows, without flow focusing, was also reported in Erbacher. Erbacher at Abstract, 21.

4436. As another example, Bio-Rad relies on a statement from Srisa-Art, M., et. al., “High-Throughput DNA Droplet Assays Using Picoliter Reactor Volumes,” *Anal. Chem.* 79:6682-6689 (2007) (“Srisa-Art”) (10X-000061162-61169):

Droplet-based microfluidic systems provide a controlled environment in which to perform rapid mixing, isolation of picoliter-size fluid volumes, and rapid variation of reaction conditions. *An elegant example of this facility has been demonstrated by Ismagilov and colleagues, in which they used droplets to measure reaction kinetics on the millisecond time scale.*

4437. As discussed above, Bio-Rad has interpreted the claims of the Ismagilov patents to extend to reaction in plugs outside of the substrate. Under such conditions, any benefits relating to “measure[ing] reaction kinetics on the millisecond time scale” are not applicable. This benefit applies only to reactions within a substrate. For example, these alleged benefits would not

apply to DNA amplification reactions outside the substrate. In fact, these alleged benefits would not apply to PCR reactions even on the substrate. In PCR reactions, the temperature is changed cyclically in time and the thermal time required to change the solution temperature to each desired value is much longer than the kinetic time scale. Even with pico-liter droplets, the cycle time is still longer than 30 seconds. Hence, the ability to measure PCR kinetics to the millisecond time scale presents no benefit in terms of optimizing the efficiency of a PCR reaction on a substrate—the speed is limited by the thermal time of the system.

4438. I understand that in a related proceeding before the Patent Office, Bio-Rad has also relied on citations to and descriptions of Roach, S., et al., “Controlling Nonspecific Protein Adsorption in a Plug-Based Microfluidic System by Controlling Interfacial Chemistry Using Fluorous-Phase Surfactants,” *Anal. Chem.* 77:785-796 (2005) (“Roach”) (10X-000255596-607), which discloses certain surfactants including a hydrophilic oligoethylene glycol head group, to support this argument. To the extent the statements highlighted by Bio-Rad constitute “praise,” these statements relate to this specific surfactant with an oligoethylene glycol head group *only*. See, e.g., IPR2015-01156 POPR at 53-58. A single asserted claim on the Ismagilov patents is directed to this surfactant, claim 9 of the ’083 patent. Accordingly, “praise” related to this specific surfactant lacks a nexus to claims with limitations relating to surfactants generally, fluorinated surfactants generally, or that do not include surfactant limitations at all. Further, as discussed above, Bio-Rad has interpreted the claims of the Ismagilov patents to extend to reaction in plugs outside of the substrate, including DNA amplification reactions in plugs outside of the substrate. Under such conditions, any benefits relating to this surfactant are not applicable. As discussed above, this surfactant would not stabilize droplets to perform a DNA amplification reaction outside of the substrate.

4439. Bio-Rad also asserts “[a]s further evidence or praise by other, RainDance’s products have been recipients of numerous awards.” I understand that Bio-Rad contends that certain of its products, including certain products previously sold by RainDance, practice certain claims of the Ismagilov patents. I have not been asked and have not formed an opinion on whether or not this is true. Regardless, Bio-Rad falls far short of satisfying what I understand is the legal requirement to show a “nexus” between this alleged “praise” and the actual features of the Ismagilov patents. This allegation focuses on the success of RainDance’s products generally, not the features of the Ismagilov patents that are allegedly practiced by the RainDance’s products.

4440. Thus, I have not seen any evidence that the features described in the Ismagilov patents received the praise of others. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

B. Long Felt But Unresolved Need

4441. I understand that Bio-Rad is contending that long felt but unresolved need is a factor that tends to show non-obviousness. Specifically, Bio-Rad has asserted that “praise” discussed above “further serves as evidence of long-felt need” because “the praise arose because the inventions of the patents-in-suit fulfilled a long-term need for a technique for conducting individualized chemical reactions on a massively parallel and miniaturized scale.” Plaintiffs’ Response to Interrogatory No. 16

4442. As an initial matter, the approach set forth in the Ismagilov patents did not fulfill any long felt but unresolved need. To the contrary, as shown in my analysis above, the allegedly inventive concepts described by the asserted patents were actually all well-known in the prior art.

4443. Further, as discussed above, Bio-Rad falls far short of satisfying what I understand is the legal requirement to show a “nexus” between this alleged “praise” and the

actual features of the Ismagilov patents. I have not seen any evidence that the features described in the Ismagilov patents received the praise of others. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

C. Failure of Others

4444. I understand that Bio-Rad is contending that “[a]n additional secondary consideration demonstrating the non-obviousness of the Asserted patents is failure of others.” Specifically, Bio-Rad has asserted that “[t]wo notable researchers . . . Todd Thorsen and Stephen Quake . . . were unsuccessful in their 2003 attempts to conduct a model enzymatic assay in droplets using their microfluidic “crossflow” system” because they addressed the wrong issue “adhesion of the cells to the channel walls and reagent stability” instead of “droplet surface” and “environments for the reagent[s].” Plaintiffs’ Response to Interrogatory No. 16.

4445. I understand that in a related proceeding before the Patent Office, Bio-Rad relied on Thorsen, T., “Microfluidic Technologies for High-Throughput Screening Applications,” Doctoral Thesis, California Institute of Technology (2002)) (“Thorsen Thesis”) to support this argument. I have reviewed the Thorsen Thesis. The Thorsen Thesis does not show a failure of others. Thorsen describes “a few good examples of catalysis inside formed droplets” and explains that a number of crossflow devices were discarded “in attempt to discover the perfect operating conditions” Thorsen at 100, 102.⁶⁹ This is a successful proof-of-concept demonstration of the potential of the cross-flow or T-junction droplet technology for single-cell enzyme assays.

4446. Further, Bio-Rad mischaracterizes the “issues” Thorsen experienced. In Thorsen’s system:

⁶⁹ As one of the “main advantages” of the microfluidic devices is “disposability,” *id.* at 148, such disposal does not indicate a failure.

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

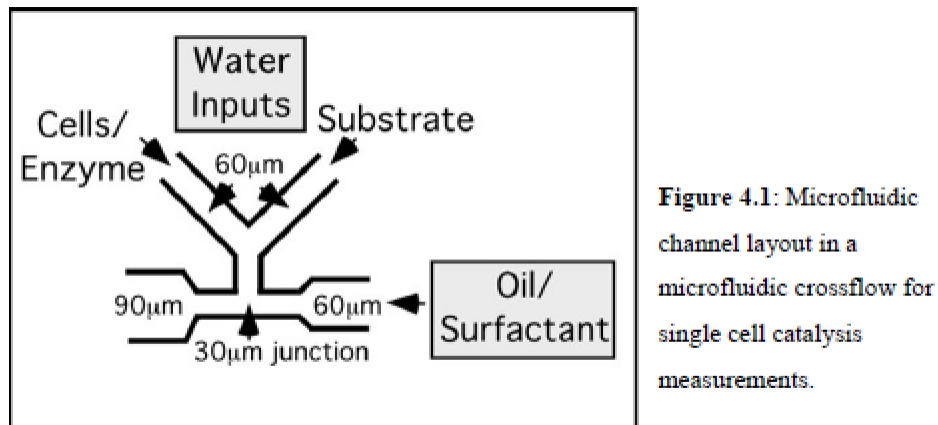


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

4447. Despite the “unreliabil[ity]” of the crossflow assay, “[a] few good examples of catalysis inside the formed droplets were still acquired (Figure 4.5).” Thorsen Thesis at 100.

4448. Thorsen describes one of the issues that required “troubleshooting” as involving “bacterial cell adhesion to the walls of the Slygard 184 microchannels *prior to encapsulation*.” *Id.* at 104 (emphasis added). To address this issues “modifications were made to both the cell resuspension solution and the PDMS surface.” *Id.* at 105. This issue of “cell adhesion” pertains to adhesion of the bacterial cells to the channel walls while the cells are in the aqueous stream *prior to encapsulation*. *Id.* at 104-105. As this cell adhesion problem occurs prior to encapsulation—*i.e.*, upstream of droplet generation at the T-junction—the “droplet surface” is

irrelevant to addressing the issue. Cell adhesion prior to encapsulation prevents equal spacing of the cells and orderly encapsulation. *Id.* at 104-107. While, this technical challenge renders Thorsen's assay less efficient, it does not render them failures.

4449. Further, the Ismagilov patents do not provide a solution to this issue. In fact, high-efficiency encapsulation of single cells into single droplets remains difficult. Agresti, J., et al., "Ultrahigh-throughput screening in drop-based microfluidics for directed evolution," *Proc. Natl. Acad. Sci.*, 107:4004-4009 (2010) ("Agresti") (10X-000254871-78); Hu, R., et al., "Encapsulation of single cells into monodisperse droplets by fluorescence-activated droplet formation on a microfluidic chip," *Talanta*, 153:253-259 (2016) ("Hu") (10X-000255348-54). Accordingly, Bio-Rad falls far short of satisfying what I understand is the legal requirement to show a "nexus" between this alleged "failure of others" and the actual features of the Ismagilov patents.

4450. This factor accordingly fails to support the notion that the Ismagilov patents are non-obvious.

D. Commercial Success

4451. Bio-Rad also appears to be contending that "commercial success" also demonstrates non-obvious, although this allegation is less than clear and Bio-Rad does not explicitly list "commercial success" as a "secondary consideration[]" [that] demonstrate[s] non-obviousness." Plaintiffs' Response to Interrogatory No. 16. Specifically, Bio-Rad has asserted that a "secondary consideration is the interest that third-parties have shown in purchasing RainDance's instruments practicing the Asserted Patents and/or RainDance's interest in the Asserted Patents" and provides the specific example that Bio-Rad purchased "RainDance on the strength of its instruments incorporating the Asserted Patents, and its instruments incorporating the Asserted Patents, and has since assumed RainDance's licenses of the Asserted Patents."

Plaintiffs' Response to Interrogatory No. 16 (citing RDTX00024277).

4452. Bio-Rad's allegations fall far short of satisfying what I understand is the legal requirement to show a "nexus" between any alleged commercial success and the actual features of the Ismagilov patents. Bio-Rad does not provide evidence that its acquisition was related to the Ismagilov patents or features in RainDance's products that are described in the Ismagilov patents. Nor does Bio-Rad provide evidence of any actual value for the Ismagilov patents or features in RainDance's products that are described in the Ismagilov patents. Further, I understand that RainDance had limited commercial success with its products, Patel Tr. 79:12-80:1, and RainDance's products are not being sold by Bio-Rad. DiPanfilo 167:1-169:20. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

E. Skepticism By Experts

4453. Another secondary consideration is "skepticism by experts." I understand that Bio-Rad is not seeking to rely of this secondary consideration and has not established a "nexus" between any "skepticism by experts" and the actual features of the Ismagilov patents. Nor do I believe that experts were or would have been skeptical of the features described in the Ismagilov patents. To the contrary, as shown in my analysis above, the allegedly inventive concepts described by the Ismagilov patents were actually all well-known in the prior art. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

F. Teaching Away By Others

4454. Another secondary consideration is "teaching away by others." I understand Bio-Rad is not seeking to rely on this secondary consideration and has not established a "nexus" between any "skepticism by teaching away by others" and the actual features of the Ismagilov patents. Nor have I seen any evidence to suggest that others taught away from the Ismagilov patents. To the contrary, as shown in my analysis above, the allegedly inventive concepts

described by the Ismagilov patents were actually all well-known in the prior art. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

G. Recognition of a Problem

4455. Another secondary consideration is “recognition of a problem.” I understand Bio-Rad is not seeking to rely on this secondary consideration and has not established a “nexus” between any “recognition of a problem” and the actual features of the Ismagilov patents. Nor have I seen any evidence to suggest that the Ismagilov patents solved a recognized problem.

4456. Moreover, I do not believe that the approach set forth in the Ismagilov patents solved a recognized problem. To the contrary, as shown in my analysis above, the allegedly inventive concepts described by the Ismagilov patents were actually all well-known in the prior art. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

H. Copying

4457. Another secondary consideration is “copying.” I understand Bio-Rad is not seeking to rely on this secondary consideration and has not established a “nexus” between any “copying” and the actual features of the Ismagilov patents. Nor have I seen any evidence to suggest that the Ismagilov patents were copied. In fact, as discussed above, Ismagilov appears to have copied from Quake. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

I. Unexpected Results

4458. Another secondary consideration is “unexpected results.” I understand Bio-Rad is not seeking to rely on this secondary consideration and has not established a “nexus” between any “unexpected results” and the actual features of the Ismagilov patents. Nor have I seen any evidence to suggest the approach set forth in Ismagilov patents demonstrated unexpected results.

4459. Moreover, I do not believe that the approach set forth in the Ismagilov patents demonstrated unexpected results. To the contrary, as shown in my analysis above, the allegedly inventive concepts described by the Ismagilov patents were actually all well-known in the prior art. This factor accordingly fails to support the notion that the Ismagilov patents are non-obviousness.

J. Simultaneous Invention

4460. I understand that evidence of “simultaneous invention” can be secondary indicia tending to show obviousness. That is evidence of others who were performing methods or creating microfluidic systems in accordance with the claimed inventions of the Ismagilov patents may serve as an objective indicia of obviousness if such evidence originates from around the time of the alleged invention (or even after). In this case, my opinion is that this evidence is compelling indicia of obviousness of the Ismagilov patents.

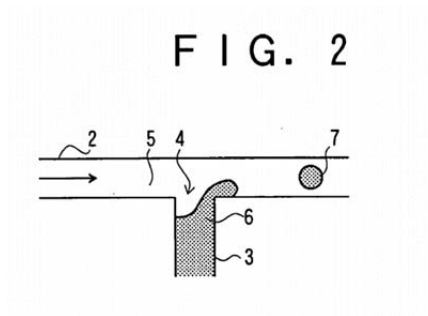
4461. As shown in my analysis above, the allegedly inventive concepts described by the Ismagilov patents were actually all well-known in the prior art.

4462. Furthermore, to the extent that Bio-Rad contends that any of the prior art references cited or relied upon in this report actually came after the date of the invention for any of the Ismagilov patents, that prior art would still constitute evidence of simultaneous invention for the same reasons set forth above. For example, I understand that a number of the prior art references that I rely upon were not published more than a year before the effective filing date, and therefore Bio-Rad could theoretically attempt to “swear behind” those references. Examples of these references include: Quake, Burns (2001), Nisisako, Seki, Curcio, and Anderson.

4463. As another example, in early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the claims of the Ismagilov patents. *See* Higuchi I-III. I understand that a patent application describing this work was filed on February 23, 2001

and was published in a patent application, Higuchi I. I understand that this application does not qualify as prior art to the Ismagilov patent because it was first published in Japanese rather than English.

4464. Higuchi I discloses a microreactor in which a dispersed phase (6) is ejected from a dispersed phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersed phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersed phase (6). Higuchi I at Fig. 2.



4465. This microchannel device was specifically intended to be used to perform emulsion-based chemical reactions. *See* Taniguchi. Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

4466. As another example, in early 2002 Todd Thorsen developed a droplet reactor that falls within the claims of the Ismagilov patents. Thorsen Thesis at 94-108. As discussed, above the Thorsen Thesis describes the following microfluidic system:

Cells expressing a recombinant enzyme and the appropriate substrate are injected into separate water channels that meet at the crossflow junction (Figure 4.1). As soon as the two water streams merge, they are immediately encapsulated into a

droplet in the oil-surfactant stream. As the droplets flow down the channel toward the outlet, the substrate is converted to a detectable fluorescent product. Under monodisperse droplet generating conditions, a PMT-based detector system can be used not only to compare endpoint activity between individual droplets at a fixed position in the outflow channel, but also to obtain single cell kinetic data for an enzyme population by taking measurements of droplets at multiple channel positions.

Thorsen Thesis at 95-96. This system is depicted in Figure 2.1 of the Thorsen Thesis:

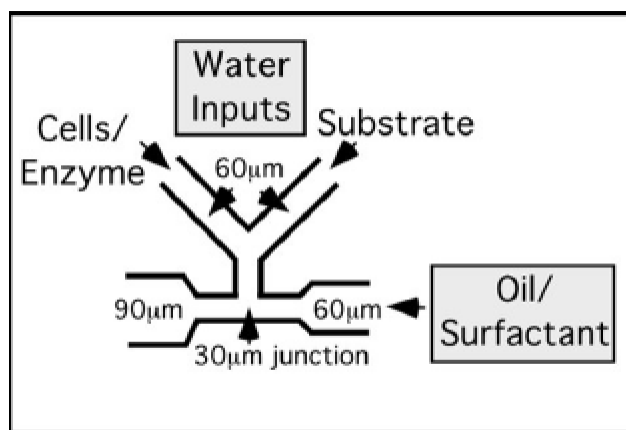
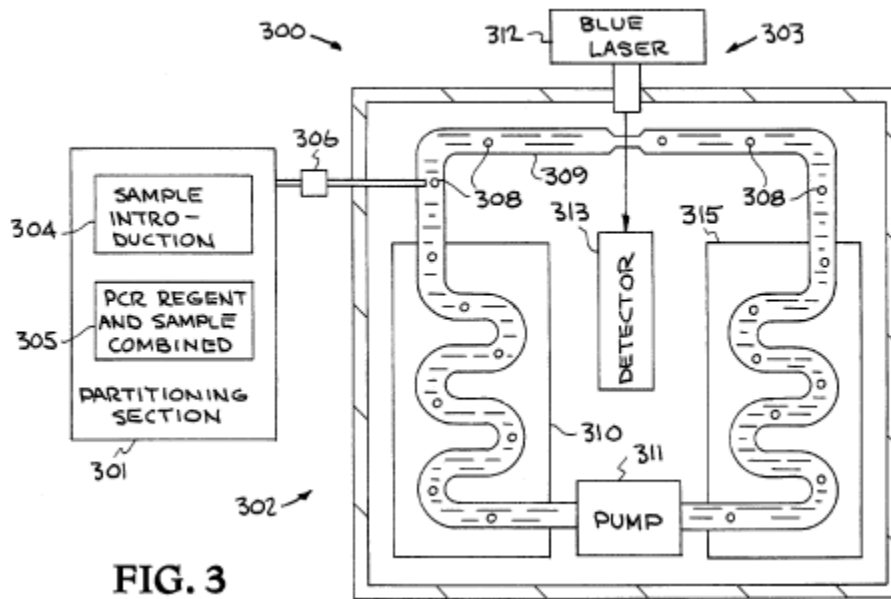


Figure 4.1: Microfluidic channel layout in a microfluidic crossflow for single cell catalysis measurements.

Despite the “unreliabil[ity]” of the crossflow assay, “[a] few good examples of catalysis inside the formed droplets were still acquired (Figure 4.5).” Thorsen Thesis at 100. The Thorsen Thesis was published and made available on December 2, 2002. However, the thesis was defended on September 23, 2002 and the “Acknowledgements” section is dated April 2002 suggesting that the work was performed before this date. Thus, the Thorsen Thesis demonstrates that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the priority date of the Ismagilov patents.

4467. As another example, in 2003, Brian Anderson, Billy Colston, and Chris Elkin developed “[a] system for nucleic acid amplification of a sample compris[ing] partitioning the sample into partitioned sections and performing PCR on the partitioned sections of the sample.”

Anderson at Abstract. Figure 3 of Anderson is reproduced below:



4468. The system depicted in Figure 3 “includes a partitioning section **301**, a PCR section **302**, and a detection and analysis section **303**.” *Id.* at 6:39-40.

The partitioning section **301** includes a sample introduction unit **304** and a unit **305** where the sample and a PCR reagent are combined. The sample and a PCR reagent are injected through a small orifice **306**. The injection of the sample through the small orifice **306** produces microdroplets **308**.

The PCR section **302** includes a continuous tube **309** for circulating the microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are pumped through the continuous tube **309** by pump **311**. The microdroplets **308** suspended in an immiscible carrier fluid **314** are cycled through heater **310** and cooler **315** to perform PCR.

The detection and analysis section **303** includes a blue laser **312** and a detector **313**. The laser **312** is projected upon the droplets **308** as they pass through tube **309** between the laser **312** and the detector **313**.

In the system **300**, the DNA-containing solution is partitioned into many microdroplets **308** and suspended in an immiscible carrier fluid **314**. The microdroplets **308** are formed by forcing the PCR mix (sample and reagent) through the small orifice or microjet **306**. These microdroplets **308** are then captured in the immiscible fluid **314**, such as mineral oil, and flowed past the heating element **310** and cooler **315**. An optical signal (e.g., fluorescence or optical absorption), generated by degradation of the dye/quencher pair on the primer, is detected using a confocal imaging system such as that employed in conventional flow cytometers. Scattering profiles from individual microdroplets, as in conventional flow cytometers, can be used to eliminate background signal from other particles. Once exposed to multiple heating cycles, the microdroplets can be identified and probed for an optical signal at rates of several thousand per second.

Id. at 6:41-7:8.

4469. “[E]ach partitioned DNA containing fluid volume contains the necessary biochemical constituents for selectively amplifying a specified portion of a sample DNA via polymerase chain reaction (PCR).” *Id.* at 7:47-50. Further, “given the extremely small volume” of the system “it is possible to isolate a single template of the target DNA in a given partitioned volume or microdroplet.” *Id.* at 7:34-37. Thus Anderson demonstrates that the use of microdroplet reactors to perform PCR was within the level of skill in the art as of the priority date of the Ismagilov patents.

XV. CONCLUSION

4470. It is my opinion that the asserted claims of the Ismagilov patents are invalid for the reasons set forth above.

4471. I reserve the right to amend or supplement this report based on further preparation in this action, including my review of any further expert statements or reports submitted on behalf of Bio-Rad. I reserve the right to supplement or amend my opinions in response to

opinions expressed by Bio-Rad experts, or in light of any additional evidence, testimony, or other information that may be provided to me after the date of this report, including at trial. In addition, I expect that I may be asked to testify in rebuttal as to issues that may be raised in the report(s) presented by Bio-Rad, or to issues that may be raised by fact witnesses and technical experts at trial.

Executed on August 21, 2017, at Notre Dame, Indiana.

I declare under penalty of perjury that the foregoing is true and correct.



Prof. Hsueh-Chia Chang

EXHIBIT D

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BIO-RAD LABORATORIES, INC. and
THE UNIVERSITY OF CHICAGO,

Plaintiffs,

v.

10X GENOMICS, INC.,

Defendant.

C.A. No. 15-152-RGA

JURY TRIAL DEMANDED

EXPERT REPORT OF NICHOLAS P. GODICI

RICHARDS, LAYTON & FINGER, P.A.
Frederick L. Cottrell, III (#2555)
Jason J. Rawnsley (#5379)
920 North King Street
Wilmington, DE 19801
(302) 651-7700
cottrell@rlf.com
rawnsley@rlf.com

Of Counsel:

David I. Gindler (dgindler@irell.com)
Andrei Iancu (aiancu@irell.com)
Lauren N. Drake (ldrake@irell.com)
Elizabeth C. Tuan (etuan@irell.com)
IRELL & MANELLA LLP
1800 Avenue of the Stars, Suite 900
Los Angeles, CA 90067-4276

Michael H. Strub
Dennis J. Courtney
IRELL & MANELLA LLP
840 Newport Center Drive, Suite 400
Newport Beach, CA 92660
(949) 760-0991

Attorneys for 10X Genomics, Inc.

Dated: August 21, 2017

I. INTRODUCTION

1. I, Nicholas P. Godici, have been retained for 10X Genomics, Inc. (“10X”) as an expert witness in connection with the above-referenced matter. I have been asked to provide expert testimony on practices and procedures governing the filing and prosecution of patent applications and in the United States Patent and Trademark Office (“USPTO” or “PTO”). I have also been asked to review the prosecution histories of U.S. Patent Nos. 7,129,091 (the “’091 patent”), 8,304,193 (the “’193 patent”), 8,329,407 (the “’407 patent”), 8,822,148 (the “’148 patent”), and 8,889,083 (the “’083 patent”), which I understand to be currently asserted against 10X in the above-referenced matter, as well as U.S. Patent No. 8,273,573 (the “’573 patent”), which I understand was originally asserted against 10X in the above-referenced matter, but has since been withdrawn. I will refer to these patents as the “Ismagilov patents.”

2. I reserve the right to give opinions on facts and other matters arising subsequent to this report, including rebuttal to any matter raised by the parties or their experts, either prior to or during any hearing or trial in this action.

A. Qualifications

3. I am currently an independent patent consultant based in Carlsbad, California.

4. I have over 44 years of experience in the patent field. I spent 33 years of my career employed at the PTO as well as over 8 years as the Executive Advisor at the intellectual property law firm of Birch, Stewart, Kolasch & Birch LLP.

5. I began my career at the PTO in 1972 as a patent examiner and held the positions of Supervisory Patent Examiner, Group Director, Deputy Assistant Commissioner, and Acting Assistant Commissioner for Patents before being named Commissioner for Patents by the Secretary of Commerce on March 29, 2000. During my time as an examiner at the PTO, I examined several thousand patent applications. My name appears as the assistant or primary

examiner on over 3,700 U.S. patents. During my career in the PTO, I served as a lecturer in the PTO "Patent Academy" training hundreds of examiners on the PTO practices and procedures for examining patent applications. Additionally, as a primary examiner and supervisory patent examiner, I provided one-on-one training to dozens of examiners regarding PTO examination procedures.

6. As Commissioner for Patents, I was the Chief Operating Officer responsible for the management and direction of all aspects and activities of patent operations at the PTO. My responsibilities included the establishment and implementation of all patent examination policies, regulations, and guidelines as well as execution of a budget of over \$750 million and management of a staff of over 5,000 employees.

7. I also served as the Acting Under Secretary of Commerce for Intellectual Property and Director of the PTO from January to December 2001. In that capacity, I reported directly to and advised the Secretary of Commerce on all intellectual property matters and was responsible for all operational aspects of the PTO. I have testified before Congressional committees of both the House and Senate on various PTO related issues. I retired from the PTO in 2005.

8. After my retirement from the PTO, I held the position of Executive Advisor for the intellectual property law firm of Birch, Stewart, Kolasch & Birch LLP from April 2005 to September 2013. My responsibilities included advising clients on PTO regulations and procedures, assisting attorneys in patent prosecution matters, consulting on patent related matters, and acting as an expert witness on PTO related issues.

9. I returned to the PTO to serve on a temporary assignment from July through September 2009, at the request of the Secretary of Commerce. During that assignment, I served

as an expert advisor to the Secretary on PTO management issues, prior to Senate confirmation of the new Under Secretary nominated by the President.

10. I have numerous professional affiliations and have earned numerous awards and honors through the course of my professional career. For example, I was named “one of the most important people in intellectual property today” by Legal Times Magazine in 2001. I was also the 2010 recipient of the Pasquale J. Federico Memorial Award recognizing outstanding contributions to the Patent and Trademark Systems of the United States of America. In addition, I have been a member of the American Intellectual Property Law Association Board of Directors since 2014 and I have also served as the Chair or Vice Chair of the Patent Law Committee, The Patent Agents Committee and the Patent Relations with the USPTO Committee.

11. I hold a Bachelor of Science degree in Engineering Mechanics from Pennsylvania State University and a Certificate of Advanced Public Management from The Maxwell School of Citizenship and Public Affairs, Syracuse University. My curriculum vitae is attached as **Exhibit A** to this report, which includes a list of all publications authored by me in the previous 10 years. I may testify with respect to my responsibilities and experiences relating to the information listed in **Exhibit A**.

B. Prior Testimony

12. A list of cases in which I have testified as an expert at trial or by deposition in the last four years is attached as **Exhibit B**.

C. Materials Considered

13. The list of materials that I have considered in whole or in part in reaching my conclusions is attached as **Exhibit C**. I reserve the right to supplement and/or modify my analysis and the resulting conclusions if additional relevant information becomes available. At trial, deposition, or in any supplemental report, I may use all or a portion of the documents I

considered in preparing this report and possibly other visual aids, charts, or exhibits, which have not yet been created.

D. Compensation

14. I am being compensated for my time spent on this matter at the billing rate of \$750 per hour. The compensation received from this matter is not contingent upon my opinions or performance, the outcome of the case, or any issues involved in or related to the case.

E. Scope of My Opinions

15. At the present time, I expect to testify, both at deposition (if requested) and at trial, on (1) the practices and procedures governing the filing and prosecution of patent applications at the PTO; (2) the prosecution histories of the Ismagilov patents; and (3) the opinions expressed in the remainder of my report.

16. The opinions stated in this report are based on information currently available to me. I reserve the right to continue my investigation and study, which may include a review of documents and information that may yet be produced, including expert reports, as well as any testimony from depositions yet to be taken in this case. Therefore, I reserve the right to expand or modify this report as my investigation and study continues, and to supplement my opinions in response to any additional information that becomes available to me, any matters raised by the parties, and/or other opinions provided by the parties' expert(s). In my testimony, I may use exhibits and demonstratives.

17. Below is a high level summary of the opinions expressed in detail throughout the remainder of this report:

- The applicants on the asserted patents copied significant portions of a prior art reference into their application. The applicants never brought this fact to the attention of the examiner.

- The amount of copying, and the placement of the copied materials (in the “detailed description of the invention” and specific purported embodiments of the invention) is highly unusual.
- In my opinion, the applicants’ conduct with regard to its copying and non-disclosure was a breach of the duties of candor and good faith owed to the PTO. This conduct includes, for example, copying the Quake reference and identifying it as an embodiment of the applicants own invention, failing to disclose to the examiner that significant portions of the specification were copied from the prior art, failing to explain to the examiner that certain passages related to rejections issued by the examiner were copied from the prior art, and failing to disclose the applicants own knowledge of the copied prior art to the examiner.
- The PTO rules and procedures would have required the examiner to take additional and/or different actions during prosecution, if the examiner had been made aware of the copying. For example, if the examiner had known about the copying, the examiner would likely have imposed additional requirements on, and requested additional information from, the applicants to clearly identify how the applicants’ work differed, if at all, from the copied prior art.
- I understand from Dr. Chang¹, a technical expert in this matter, that there are technical reasons (for example anticipation by the copied prior art) that the

¹ I spoke with Dr. Chang to discuss his opinions, and Dr. Chang pointed me to relevant sections of his report that include his analysis. I further reviewed Dr. Chang’s report to confirm my understanding of our discussions.

examiner would not have allowed the claims over the copied prior art if the examiner had full knowledge of the copying.

II. OVERVIEW OF THE PATENT SYSTEM AND THE PROCEDURES GOVERNING THE FILING AND PROSECUTION OF PATENT APPLICATIONS AT THE PTO

A. The Patent System in General

18. The patent statute is found in Title 35 of the United States Code. The PTO promulgates regulations found in Title 37 of the Code of Federal Regulations that implement the patent statutes, and the PTO publishes the Manual of Patent Examining Procedures (“MPEP”) to provide patent examiners, applicants, attorneys, agents, and representatives with a reference that sets forth practices and procedures relative to the prosecution of patent applications before the PTO.²

B. Patent Application Filing Requirements

19. An applicant for a patent in the United States initiates the process by filing an application with the PTO detailing the subject matter for which patent protection is sought. The application is required to include a specification including a claim or claims, drawings when necessary, an inventor’s oath or declaration, and the required fees.³

20. The specification of an application will normally include the following sections separated by headings: a title of the invention, cross-reference to related applications, the background of the invention, a brief summary of the invention, brief description of the drawings, detailed description of the invention, and an abstract of the disclosure.⁴ The background section

² See the Foreword to the MPEP.

³ 35 U.S.C. § 111(a).

⁴ 37 C.F.R. 1.77(b).

describes the field of the invention and the state of the prior art.⁵ The detailed description of the invention describes what the applicants regard as their invention.⁶

21. The specification must conclude with a set of one or more claims. The claims must particularly point out and distinctly describe the subject matter that the applicant regards as the invention.⁷ The claims are an important part of the application or patent because they define the metes and bounds of the protection the applicant is seeking and serve to inform the public of the scope of patent protection in a granted patent.

22. Claims may be written in independent or dependent form. An independent claim stands by itself and does not refer to or incorporate any other claim. A dependent claim, on the other hand, refers to another claim and incorporates and further limits that claim.⁸

23. The oath or declaration made by the inventor(s) certifies that the inventor believes that he or she is the original first inventor (or joint inventor if applicable) of the subject matter claimed and for which a patent is sought and that he or she has reviewed and understands the contents of the application including the specification and claims. It is improper to attempt to claim an invention derived from subject matter of another.⁹ The inventor also makes various other certifications required by law and various PTO rules including the duty of disclosure which requires the inventor(s) to disclose information material to patentability, and a duty of candor to the Patent Office, as more fully disclosed below.

⁵ MPEP § 608.01(e).

⁶ MPEP § 608.01(g).

⁷ 37 C.F.R. § 1.75(a).

⁸ 37 C.F.R. § 1.75(c).

⁹ 35 U.S.C. 102 (f).

C. Substantive Examination

24. After a patent application is received, administrative staff for the PTO performs a preliminary review to ensure that an application meets the formal (administrative) requirements established by Congress and the PTO. If the application fails to meet the formal requirements, the applicant will be notified of the deficiencies and given a time period in which to complete the formal requirements of the application.¹⁰

25. After the review of a patent application for formal matters, the application is assigned to an examiner in a Group Art Unit that is responsible for examining the substance of patent applications in a particular technological area.

26. In the examination of a patent application, an examiner is required to determine whether the claims of the application satisfy all of the requirements for patentability set forth in the patent statutes and regulations, as more fully described below. Although the PTO strives to conduct high quality examinations, an examiner's time and resources are limited. An examiner's performance is evaluated based on the quantity, quality, and timeliness of the examiner's work. Quantity is measured using a production or quota system. An examiner's individual production goal is based on the difficulty of the technology of the applications assigned to the examiner and the grade or experience level of the examiner. Given the limited time and resources available for examination, examiners simply cannot independently verify each and every fact or argument presented by an applicant. For these reasons (among others), examiners rely on an applicant's duty of candor and good faith when filing and prosecuting their patent application. As explained in further detail below, here the applicants breached their duty of candor and good faith. This improper conduct includes, for example, copying portions of the Quake reference and identifying

¹⁰ MPEP § 601.01 (a) II B.

it as an embodiment of the applicants own invention, failing to disclose to the examiner that significant portions of the specification were copied from the prior art, failing to explain to the examiner that certain passages related to rejections issued by the examiner were copied from the prior art, failing to disclose the applicants own knowledge of the copied prior art to the examiner, and failing to disclose rejections made by a different examiner in a related application over the very same copied prior art.

27. As stated above, the PTO issues a set of procedures and guidelines, called the Manual of Patent Examination Procedure (MPEP), that govern the patent examination process. Among other uses, the MPEP is provided to patent examiners as a basic “guide-book” for how examiner’s should conduct their examination. The MPEP contains the patent statutes, rules promulgated by the PTO, procedures, and official PTO positions on the interpretation of controlling law. Examiners will consult the MPEP to determine the correct course of action depending on how the prosecution of an application progresses and the facts that come before the examiner. For example, the MPEP may specify that a certain rejection must be made in response to a certain fact disclosed by the applicant. Or it may specify that an examiner should seek certain additional information from an applicant in view of specific prior art or arguments advanced by the applicant, or other appropriate circumstances. Fundamentally, the MPEP sets forth the procedures to be followed during the examination process.

28. The examination of claims in a patent application also requires an examiner to conduct a search for “prior art” related to the claimed invention.¹¹ Prior art may include prior patents (both U.S. patents and foreign patents), prior published patent applications, and prior non-patent publications (e.g., magazines, trade journals, and published academic theses)

¹¹ MPEP § 704.01.

sometimes called non-patent literature.¹² In addition, prior art may include prior public uses, sales, and offers for sale of the claimed invention in this country more than one year prior to the filing date of an application.¹³ “Prior art” may also include other activities, such as certain foreign filings,¹⁴ statements of an inventor expressing an intent to “abandon” his invention,¹⁵ or facts related to derivation of the invention from another,¹⁶ for example.

29. While applicants are not required to conduct a prior art search, they are required to disclose material information that they are aware of to the PTO under their duty of candor and good faith and their duty of disclosure. “Material information” is not limited to prior art documents, such as patents and publications. Instead, “Material information” includes any information material to the patentability of the claims under examination. Many applicants disclose information they are aware of to the PTO via an Information Disclosure Statement (IDS). If an IDS is filed in compliance with PTO regulations, the examiner will consider the prior art references or other information in the IDS during the examination of the application.

30. After analyzing the prior art and any other issues, the examiner issues an Office Action stating his or her conclusions with respect to patentability. In addition to the prior art, the examiner will analyze the applicant’s specification to understand specific examples of embodiments of the invention, the broadest reasonable scope of the claimed invention, and other important features of the disclosure of the application. An Office Action informs the applicant

¹² 35 U.S.C. § 102.

¹³ 35 U.S.C. § 102 (b).

¹⁴ 35 U.S.C. § 102 (d).

¹⁵ 35 U.S.C. § 102 (c).

¹⁶ 35 U.S.C. § 102 (f).

whether the examiner has found some, all, or none of the claims to be patentable in their current state, and will explain the reasons for the examiner's conclusions.

31. Under certain circumstances, a later filed application for patent is entitled to the benefit of the filing date of a prior filed or parent application.¹⁷ One type of continuing or "child" application is a continuation application. A continuation is a second application for the same invention claimed in a prior application and filed before the original application becomes abandoned or patented.¹⁸

32. Another type of continuing or "child" application is a divisional application. A divisional application is a later filed application for an independent and distinct invention carved out of a parent application. A divisional application is often filed as a result of a restriction requirement, which is made by the PTO if it is determined that an application contains claims to more than one independent and distinct invention.¹⁹

33. An applicant may also claim the benefit of the filing date of an earlier filed provisional application under 35 U.S.C. § 119(e) provided that the nonprovisional application is filed within 12 months after the filing date of the provisional application. Provisional applications are not examined and an examiner will not generally review a provisional patent application unless there is an issue with the priority date, for example, based on prior art dated before the filing of the non-provisional under review, but after the provisional filing date.

¹⁷ See MPEP § 201.11.

¹⁸ See MPEP § 201.07.

¹⁹ See MPEP § 201.06.

D. The Requirements for Patentability

34. For an invention to be patentable, the claims must, among other things, set forth subject matter that is eligible for patenting, be novel and nonobvious in view of the prior art, and be sufficiently described to enable one of ordinary skill in the art to make and use the invention. The PTO is charged with evaluating the claims of a patent application to determine if all the statutory requirements for patentability are met.

35. When examining a patent application, a patent examiner considers all of these requirements. If the patent examiner determines that any of the requirements for patentability have not been met, the examiner will reject the claims of the application.

36. Claims may be rejected, for example, because the examiner concludes that they are not novel in light of the prior art (anticipation under 35 U.S.C. § 102) or differ only in obvious ways (obviousness under 35 U.S.C. § 103) from the existing prior art. When making an obviousness rejection under 35 U.S.C. § 103, the examiner has the initial burden of factually supporting a *prima facie* conclusion of obviousness, thus shifting the burden to the applicant to respond with evidence of non-obviousness.²⁰ The references and information relied on by the examiner and the reasons for the rejection are set forth in the Office Action that is sent to the applicant.²¹

37. Claims may also be rejected if drawn to subject matter not eligible for patenting (35 U.S.C. § 101), if the specification does not contain a written description of the invention that enables one of ordinary skill in the art to make and use the invention (35 U.S.C. § 112, first

²⁰ MPEP § 2142.

²¹ 37 C.F.R. § 1.104.

paragraph), and/or if the claims fail to particularly point out and distinctly claim the invention (35 U.S.C. § 112, second paragraph).

E. Applicant's Response and Reconsideration by the Examiner

38. After receiving an Office Action containing rejections or objections, an applicant may amend the specification and/or claims in an effort to overcome the examiner's rejections. An applicant may also reply to the rejections and objections set forth in an Office Action by filing a response pointing out what the applicant believes are errors in the examiner's action. The applicants must respond to each ground of rejection or objection. Failure to do so can result in the examiner treating the reply as non-responsive and require the applicant to file a complete response.²²

39. The examiner then reviews any amendments and/or arguments submitted by the applicant to determine if the conditions for patentability are met. The examiner can either allow the application or issue a new Office Action if rejections and/or objections remain. There are typically one or more rounds of this "back and forth" between the examiner and the applicant, before prosecution on the merits is closed. If the claims in the application continue to be rejected, normally the examiner's second action is made "final." At that point, the applicant can either appeal the decision of the examiner to the PTO Patent Trial and Appeal Board (and ultimately to the federal courts), file a continuing application or request for continued examination, or abandon the application.

40. If, on the examiner's original examination of the application or after considering the responses and amendments made by the applicant, the patent application is found to be in allowable condition, a Notice of Allowance will be sent to the applicant. An examiner may

²² 37 C.F.R. § 1.111 and MPEP § 713.04.

include reasons for allowance in the file history to make clear his or her reason for allowing the claims of an application. A fee for issuing the patent is due within three months from the date of the Notice. Once the issue fee is paid, the application will issue as a patent.²³

41. Once issued, United States patents are presumed valid. This is sometimes called the “presumption of validity.”²⁴ I understand that this presumption may be overcome by clear and convincing evidence. I also understand that if the PTO did not have all material facts before it when making its decision on patentability the PTO’s judgment may lose significant force and that fact may be taken into consideration when a jury considers an invalidity defense.²⁵

F. The Duty of Candor

42. Patent prosecution is an “ex parte” process between the applicants or their attorneys and the USPTO examiner. Third party participation is not permitted. As a result, patent applicants, their representatives, and others substantively involved in prosecution of a patent application have a duty of candor and good faith in dealing with the PTO. Additionally, these individuals have a duty to provide information known to be material to patentability of the claims pending in the application. This obligation, which is referred to as the applicant’s “duty of

²³ MPEP § 1309.

²⁴ 35 U.S.C. § 282.

²⁵ See *Microsoft v. i4i*, 131 S.Ct. 2238, 2251 (2011) (“Simply put, if the PTO did not have all material facts before it, its considered judgment may lose significant force. *Cf.* KSR, 550 U.S., at 427, 127 S.Ct. 1727. And, concomitantly, the challenger’s burden to persuade the jury of its invalidity defense by clear and convincing evidence may be easier to sustain. In this respect, although we have no occasion to endorse any particular formulation, we note that a jury instruction on the effect of new evidence can, and when requested, most often should be given. When warranted, the jury may be instructed to consider that it has heard evidence that the PTO had no opportunity to evaluate before granting the patent. When it is disputed whether the evidence presented to the jury differs from that evaluated by the PTO, the jury may be instructed to consider that question. In either case, the jury may be instructed to evaluate whether the evidence before it is materially new, and if so, to consider that fact when determining whether an invalidity defense has been proved by clear and convincing evidence.”).

disclosure” is important to the successful operation of the Patent System. The duty of candor and good faith, as stated when the Ismagilov Patents were being prosecuted, is set forth in, among other things, 37 CFR § 1.56(a) which states:

A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) Prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.²⁶

²⁶ 37 C.F.R. § 1.56 (a).

III. FACTUAL BACKGROUND

A. Quake PCT and the '927 Application

43. On March 21, 2002, the patent document WO 02/23163 A1 (“Quake PCT”) was published.²⁷ This document—a publication of an international patent application filed under the Patent Cooperation Treaty (PCT)—names Stephen R. Quake and Todd Thorsen as inventors.

44. On May 9, 2002, Rustem Ismagilov submitted Provisional Application Ser. No. 60/379,927 (the “’927 application”) to the PTO, and that each of the Ismagilov patents claims priority to the ’927 application.²⁸

45. Much of the text appearing in the ’927 application appears to have been copied from Quake PCT, frequently verbatim or near-verbatim. One example of the close correspondence between Quake PCT and the ’927 application is as follows:

Quake PCT at 23:11-14, 23:31-24:16	'927 application at 30:5-27
A variety of channels for sample flow and mixing can be microfabricated on a single chip and can be positioned at any location on the chip as the detection and discrimination or sorting points, e.g., for kinetic studies (10, 11). A plurality of analysis units of the invention may be combined in one device. . . .	A variety of channels for sample flow and mixing can be micro fabricated on the substrate and can be positioned at any location on the substrate, chip, or device as the detection and discrimination or sorting points, e.g., for kinetic studies. A plurality of analysis units of the invention may be combined in one device.
A device of the invention can be microfabricated with a sample solution reservoir or well at the inlet region, which is typically in fluid communication with an inlet channel. A reservoir may facilitate introduction of molecules or cells into the device and into the sample inlet channel of each analysis unit. An inlet region may have an opening such as in the floor of the	A device of the invention can be microfabricated with a sample solution reservoir or well at the inlet port, which is typically in fluid communication with an inlet channel. A reservoir may facilitate introduction of molecules, particles, or substances into the device and into the sample inlet channel of each analysis unit. An inlet port may have an opening such as in the floor

²⁷ Quake PCT is attached to my report as Exhibit D.

²⁸ The ’927 application is attached to my report as Exhibit G.

Quake PCT at 23:11-14, 23:31-24:16	'927 application at 30:5-27
<p>microfabricated chip, to permit entry of the sample into the device. The inlet region may also contain a connector adapted to receive a suitable piece of tubing, such as liquid chromatography or HPLC tubing, through which a sample may be supplied. Such an arrangement facilitates introducing the sample solution under positive pressure in order to achieve a desired pressure at the droplet extrusion region.</p> <p>A device of the invention may have an additional inlet region, in direct communication with the main channel at a location upstream of the droplet extrusion region, through which a pressurized stream or "flow" of a fluid is introduced into the main channel. Preferably, this fluid is one which is not miscible with the solvent or fluid of the sample. For example, most preferably the fluid is a non-polar solvent, such as decane (<i>e.g.</i>, tetradecane or hexadecane), and the sample (<i>e.g.</i>, of cells, virions or molecules) is dissolved or suspended in an aqueous solution so that aqueous droplets of the sample are introduced into the pressurized stream of non-polar solvent at the droplet extrusion region.</p>	<p>of the microfabricated substrate, chip, or device, to permit entry of the sample into the device. The inlet port may also contain a connector adapted to receive a suitable piece of tubing, such as liquid chromatography or HPLC tubing, through which a sample may be supplied. Such an arrangement facilitates introducing the sample solution under positive pressure in order to achieve a desired pressure at the plug-forming region.</p> <p>A device of the invention may have an additional inlet port, in direct communication with the first channel at a location upstream of the plug-forming region, through which a pressurized stream or fluid flow is introduced into the first channel. Preferably, this fluid is one which is not miscible with the solvent or sample fluid. For example, most preferably the fluid is a non polar solvent, such as decane (<i>e.g.</i>, tetradecane or hexadecane), and the sample (<i>e.g.</i>, of molecules, particles, or substances) is dissolved or suspended in an aqueous solution so that aqueous plugs of the sample are formed upon coming in contact with a stream of non-polar solvent at the plug-forming region.</p>

46. Much of this language was incorporated into and appears again in the issued

Ismagilov patents:

Quake PCT at 23:11-13, 23:31-24:16	Ismagilov patents ²⁹
A variety of channels for sample flow and mixing can be microfabricated on a single chip and can be positioned at any location on the chip as the detection and discrimination or sorting points . . .	A variety of channels for sample flow and mixing can be micro fabricated on the substrate and can be positioned at any location on the substrate, chip, or device as the detection and discrimination or sorting points.

²⁹ '091 patent at 17:10-13, 16:48-59; '193 patent at 17:3-6, 16:41-52; '407 patent at 17:47-50, 17:18-29; '573 patent at 18:10-13, 17:48-59; '148 patent at 17:3-6, 16:41-52; '083 patent at 16:50-53, 16:20-31.

Quake PCT at 23:11-13, 23:31-24:16	Ismagilov patents ²⁹
<p>A device of the invention can be microfabricated with a sample solution reservoir or well at the inlet region, which is typically in fluid communication with an inlet channel. A reservoir may facilitate introduction of molecules or cells into the device and into the sample inlet channel of each analysis unit. An inlet region may have an opening such as in the floor of the microfabricated chip, to permit entry of the sample into the device. The inlet region may also contain a connector adapted to receive a suitable piece of tubing, such as liquid chromatography or HPLC tubing, through which a sample may be supplied. Such an arrangement facilitates introducing the sample solution under positive pressure in order to achieve a desired pressure at the droplet extrusion region.</p>	<p>A substrate can be fabricated with a fluid reservoir or well at the inlet port, which is typically in fluid communication with an inlet channel. A reservoir preferably facilitates introduction of fluids into the substrate and into the first channel. An inlet port may have an opening such as in the floor of the substrate to permit entry of the sample into the device. The inlet port may also contain a connector adapted to receive a suitable piece of tubing, such as Teflon® tubing, liquid chromatography or HPLC tubing, through which a fluid may be supplied. Such an arrangement facilitates introducing the fluid under positive pressure in order to achieve a desired pressure at the plug-forming region.</p>

47. Another example of the close correspondence between Quake PCT and the '927 application is as follows:

Quake PCT at 35:9-24 and 36:29-37:4	'927 application at 12:8-21, 22:21-28
<p>The droplet forming liquid is typically an aqueous buffer solution, such as ultrapure water (e.g., 18 mega-ohm resistivity, obtained, for example by column chromatography), 10 M Tris HCl and 1 mM EDTA (TE) buffer, phosphate buffer saline (PBS) or acetate buffer. Any liquid or buffer that is physiologically compatible with the population of molecules, cells or virions to be analyzed and/or sorted can be used. The fluid passing through the main channel and in which the droplets are formed is preferably one that is not miscible with the droplet forming fluid. Preferably, the fluid passing through the main channel is a non-polar solvent, most preferably decane (e.g.,</p>	<p>A plug forming liquid maybe an aqueous buffer solution, such as ultrapure water (<i>e.g.</i>, 18 mega ohm resistivity, obtained, for example by column chromatography), 10 mM Tris HCl and 1 mM EDTA (TE) buffer, phosphate buffer saline (PBS) or acetate buffer. Any liquid or buffer that is compatible with the reagents can be used.</p> <p>The fluid passing through the first channel is preferably immiscible with the plug forming fluid.</p> <p>Preferably, the fluid passing through the first channel is a non-polar solvent, most preferably an oil.</p>

Quake PCT at 35:9-24 and 36:29-37:4	'927 application at 12:8-21, 22:21-28
<p>tetradecane or hexadecane) or another oil.</p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel. . . .</p> <p>The concentration (i.e., number) of molecules, cells or virions in a droplet can influence sorting efficiently and therefore is preferably optimized. In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions. This is to ensure that for the large majority of measurements, the level of reporter measured in each droplet as it passes through the detection region corresponds to a single molecule, cell or virion and not to two or more molecules, cells or virions.</p>	<p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (<i>e.g.</i>, surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing plug size, flow and uniformity, for example by reducing the shear force needed to extrude or inject plugs into an intersecting channel. This may affect plug volume and periodicity, or the rate or frequency at which plugs break off into an intersecting channel. . . .</p> <p>The concentration of molecules, particles, or substances in a plug can influence sorting efficiently and therefore is preferably optimized. Thus, in one embodiment of the invention, the sample concentration may be adjusted to be dilute enough that most of the plugs contain no more than a single molecule or particle, with only a small statistical chance that a plug will contain two or more molecules or particles. This is to ensure that for the large majority of measurements, the level of reporter measured in each plug as it passes through the detection region corresponds to a single molecule or particle and not to two or more molecules or particles.</p>

48. Much of this language was incorporated into and appears again in the issued

Ismagilov patents:

Quake PCT at 35:9-14, 35:18-24, 36:29-37:4	Ismagilov patents ³⁰
--	---------------------------------

³⁰ '091 patent at 20:17-23, 20:45-47, 20:62-64, 20:49-55, 20:29-33; '193 patent at 20:3-9, 20:31-33, 20:47-49, 20:35-39, 20:14-19; '407 patent at 20:46-52, 21:7-15, 21:23-25, 21:12-17, 20:57-62; '573 patent at 21:12-17; 21:40-42, 21:56-58, 21:44-50, 21:23-28; '148 patent at 20:3-9, 20:31-33, 20:47-49, 20:39-41, 20:14-19; '083 patent at 19:51-57, 20:12-14, 20:29-31, 20:16-20, 19:62-57.

Quake PCT at 35:9-14, 35:18-24, 36:29-37:4	Ismagilov patents ³⁰
<p>The droplet forming liquid is typically an aqueous buffer solution, such as ultrapure water (e.g., 18 mega-ohm resistivity, obtained, for example by column chromatography), 10 M Tris HCl and 1 mM EDTA (TE) buffer, phosphate buffer saline (PBS) or acetate buffer. Any liquid or buffer that is physiologically compatible with the population of molecules, cells or virions to be analyzed and/or sorted can be used.</p> <p>The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel. This may affect droplet volume and periodicity, or the rate or frequency at which droplets break off into an intersecting channel. . . .</p> <p>The concentration (i.e., number) of molecules, cells or virions in a droplet can influence sorting efficiently and therefore is preferably optimized. In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.</p>	<p>In a preferred embodiment, the solvent may be an aqueous buffer solution, such as ultrapure water (e.g., 18 MΩ resistivity, obtained, for example, by column chromatography), 10 mM Tris HCl, and 1 mM EDTA (TE) buffer, phosphate buffer saline or acetate buffer. Other solvents that are compatible with the reagents may also be used.</p> <p>The carrier-fluid or plug-fluid, or both may contain additives, such as agents that reduce surface tensions (e.g., surfactants).</p> <p>Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.)).</p> <p>Surfactants may be used to facilitate the control and optimization of plug size, flow and uniformity. For example, surfactants can be used to reduce the shear force needed to extrude or inject plugs into an intersecting channel. Surfactants may affect plug volume or periodicity, or the rate or frequency at which plugs break off into an intersecting channel.</p> <p>The concentration of reagents in a plug can be varied. In one embodiment according to the invention, the reagent concentration may be adjusted to be dilute enough that most of the plugs contain no more than a single molecule or particle, with only a small statistical chance that a plug will contain two or more molecules or particles.</p>

49. Another example of the close correspondence between Quake PCT and the '927 application is as follows:

Quake PCT at 34:14-35:8	'927 application at 21:26-22:20
According to the invention, molecules (such as DNA, protein, enzyme or substrate) or particles (i.e., cells, including virions) are	According to the invention, molecules, particles, or substances are sorted dynamically in a flow stream of microscopic dimensions

Quake PCT at 34:14-35:8	'927 application at 21:26-22:20
<p>sorted dynamically in a flow stream of microscopic dimensions based on the detection or measurement of a characteristic, marker or reporter that is associated with the molecules or particles. More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel. The individual droplets are then analyzed and/or sorted in the flow stream, thereby sorting the molecules, cells or virions contained within the droplets.</p> <p>The flow stream in the main channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed. Prior to sorting, a liquid that does not contain samples molecules, cells or virions can be introduced into a sample inlet region (such as an inlet well or channel) and directed through the droplet extrusion region, <i>e.g.</i>, by capillary action, to hydrate and prepare the device for use. Likewise, buffer or oil can also be introduced into a main inlet region that communicates directly with the main channel to purge the device (<i>e.g.</i>, of “dead” air) and prepare it for use. If desired, the pressure can be adjusted or equalized, for example, by adding buffer or oil to an outlet region.</p> <p>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected</p>	<p>based on the detection or measurement of a characteristic, marker, property, or reporter that is associated with the molecules, particles, or substances. More specifically, plugs of a solution (preferably an aqueous solution or buffer) containing a sample of molecules, particles, or substances are introduced through a plug-forming region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the first channel. The individual plugs are then analyzed and/or sorted in the flow stream, thereby sorting the molecules, particles, or substances contained within the plugs.</p> <p>The flow stream in the first channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed. Prior to sorting, a liquid that does not contain sample molecules, particles, or substances can be introduced into a sample inlet port (such as an inlet well or channel) and directed through the plug-forming region, <i>e.g.</i>, by capillary action, to hydrate and prepare the device for use. Likewise, buffer or oil can also be introduced into a main inlet port that communicates directly with the first channel to purge the device (<i>e.g.</i>, of “dead” air) and prepare it for use. If desired, the pressure can be adjusted or equalized, for example, by adding buffer or oil to an outlet port.</p> <p>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug-forming region or the sample inlet connected thereto to control the flow of</p>

Quake PCT at 34:14-35:8	'927 application at 21:26-22:20
thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets. Periodicity and droplet volume may also depend on channel diameter, the viscosity of the fluids, and shear pressure.	solution into the plug-forming region, thereby controlling the size and periodicity of the plugs. Periodicity and plug volume may also depend on channel diameter the viscosity of the fluids, and shear pressure.

50. Much of this language was incorporated into and appears again in the issued

Ismagilov patents:

Quake PCT at 34:23-35:8	Ismagilov patents³¹
<p>The flow stream in the main channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed. Prior to sorting, a liquid that does not contain samples molecules, cells or virions can be introduced into a sample inlet region (such as an inlet well or channel) and directed through the droplet extrusion region, <i>e.g.</i>, by capillary action, to hydrate and prepare the device for use. Likewise, buffer or oil can also be introduced into a main inlet region that communicates directly with the main channel to purge the device (<i>e.g.</i>, of “dead” air) and prepare it for use. If desired, the pressure can be adjusted or equalized, for example, by adding buffer or oil to an outlet region.</p>	<p>The flow stream in the first channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed. Prior to sorting, a non-plug-fluid can be introduced into a sample inlet port (such as an inlet well or channel) and directed through the plug-forming region, <i>e.g.</i> by capillary action, to hydrate and prepare the device for use. Likewise, buffer or oil can also be introduced into a main inlet port that communicates directly with the first channel to purge the substrate (<i>e.g.</i>, of “dead” air) and prepare it for use. If desired, the pressure can be adjusted or equalized, for example, by adding buffer or oil to an outlet port.</p>
<p>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be</p>	<p>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either</p>

³¹ '091 patent at 21:60-22:16; '193 patent at 21:45-67; '407 patent at 22:21-43; '573 patent at 22:54-23:9; '148 patent at 21:45-67; '083 patent at 21:27-49.

Quake PCT at 34:23-35:8	Ismagilov patents³¹
placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets. Periodicity and droplet volume may also depend on channel diameter, the viscosity of the fluids, and shear pressure.	the plug-forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs. Periodicity and plug volume may also depend on channel diameter and/or the viscosity of the fluids.

51. Another example of the close correspondence between Quake PCT and the '927 application is as follows:

Quake PCT at 27:9-21, 27:23-32	'927 application at 10:11-30
In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa. In preferred embodiments, droplets are at least about four times as long as they are wide. This droplet configuration, which can be envisioned as a lozenge shape, flows smoothly and well through the channels. Longer droplets, produced in narrower channels, provides a higher shear, meaning that droplets can more easily be sheared or broken off from a flow, i. e. using less force. Droplets may also tend to adhere to channel surfaces, which can slow or block the flow, or produce turbulence. Droplet adherence is overcome when the droplet is massive enough in relation to the channel size to break free. Thus, droplets of varying size, if present, may combine to form uniform droplets having a so-called critical mass or volume that results in smooth or laminar droplet flow. . . . Thus, in an exemplary embodiment with 60 micron channels, a typical free-flowing droplet is about 60 microns wide and 240 microns long. Droplet dimensions and flow characteristics can be influenced as desired, in part by changing the channel dimensions, e.g. the	In one preferred embodiment, plugs conform to the size and shape of the channels while maintaining their respective volumes. Thus, as plugs move from a wider channel to a narrower channel they become longer and thinner, and vice versa preferred embodiments, plugs are at least about four times as long as the are wide. This plug configuration, which can be envisioned as a lozenge shape, flows smoothly and well through the channels. Longer plugs, produced in narrower channels, provides a higher shear, meaning that plugs can more easily be sheared or broken off from a flow, i.e. using less force. Plugs may also tend to adhere to channel surfaces, which can slow or block the flow, or produce turbulence. Plug adherence is overcome when the plug is massive enough in relation to the channel size to break free. Thus, plugs of varying size, if present, may combine to form uniform plugs having a so-called critical mass or volume that results in smooth or laminar plug flow. In an exemplary embodiment with about 50 micron channels, a typical free flowing plug is about 50 microns wide and between about 50-200 microns long. Plug dimensions and flow characteristics can be influenced as desired, in part by changing the channel shape or dimensions such as the channel width.

Quake PCT at 27:9-21, 27:23-32	'927 application at 10:11-30
channel width. More preferably, however, the microfabricated devices of this invention generate round, monodisperse droplets Monodisperse droplets may be particularly preferably, e.g., in high throughput devices and other embodiments where it is desirable to generate droplets at high frequency.	More preferably, however, the microfabricated devices of this invention generate round, monodisperse plugs. Monodisperse plugs may be particularly preferred such as in high throughput devices and other embodiments where it is desirable to generate plugs at high frequency.

52. Much of this language was incorporated into and appears again in the issued

Ismagilov patents:

Quake PCT at 27:9-12	Ismagilov patents³²
In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa.	In one preferred embodiment, plugs conform to the size and shape of the channels while maintaining their respective volumes. Thus, as plugs move from a wider channel to a narrower channel they preferably become longer and thinner, and vice versa.

53. Another example of the close correspondence between Quake PCT and the '927

application is as follows:

Quake PCT at 32:26-33:4	'927 application at 24:9-21
In a preferred embodiment, the molecules or cells or virions (or droplets containing them) are analyzed and/or separated based on the intensity of a signal from an optically-detectable reporter bound to or associated with them as they pass through a detection window or "detection region" in the device. Molecules or cells or virions having an amount or level of the reporter at a selected threshold or within a selected range are diverted into a	In a preferred embodiment, the reagent fluids, reaction products or plugs are analyzed, detected, characterized and/or separated based on the intensity of a signal from an optically detectable compound associated with them as they pass through a detection window or "detection region" in the device. Reagent fluids, reaction products or plugs having an amount or level of the reporter at a selected threshold or within a selected range are

³² '091 patent at 20:5-9; '193 patent at 19:58-62; '407 patent at 20:34-38; '573 patent at 20:66-21:3; '148 patent at 19:58-62; '083 patent at 19:39-43.

Quake PCT at 32:26-33:4	'927 application at 24:9-21
<p>predetermined outlet or branch channel of the device. The reporter signal may be collected by a microscope and measured by a photo multiplier tube (PMT). A computer digitizes the PMT signal and controls the flow via valve action or electro-osmotic potentials. Alternatively, the signal can be recorded or quantified as a measure of the reporter and/or its corresponding characteristic or marker, e.g., for the purpose of evaluation and without necessarily proceeding to sort the molecules or cells.</p>	<p>diverted into a predetermined outlet or branch channel of the device. The reporter signal may be collected by a microscope and measured by a detector such as a photo multiplier tube (PMT). A computer digitizes the PMT signal and controls the flow via methods such as valve action or electro osmotic potentials. Alternatively, the signal can be recorded or quantified as a measure of the reporter and/or its corresponding characteristic or marker, e.g., for the purpose of evaluation and without necessarily proceeding to sort the reagent fluids, reaction products or plugs.</p>

54. Much of this language was incorporated into and appears again in the issued

Ismagilov patents:

Quake PCT at 32:26-33:4	Ismagilov patents ³³
<p>In a preferred embodiment, the molecules or cells or virions (or droplets containing them) are analyzed and/or separated based on the intensity of a signal from an optically-detectable reporter bound to or associated with them as they pass through a detection window or "detection region" in the device. Molecules or cells or virions having an amount or level of the reporter at a selected threshold or within a selected range are diverted into a predetermined outlet or branch channel of the device. The reporter signal may be collected by a microscope and measured by a photo multiplier tube (PMT). A computer digitizes the PMT signal and controls the flow via valve action or electro-osmotic potentials. Alternatively, the signal can be recorded or quantified as a measure of the reporter and/or its corresponding characteristic or marker, e.g., for the purpose of evaluation and without</p>	<p>In a preferred embodiment, the plugs are analyzed based on the intensity of a signal from an optically detectable group, moiety, or compound (referred to here as "tag") associated with them as they pass through a detection window or detection region in the device. Plugs having an amount or level of the tag at a selected threshold or within a selected range can be directed into a predetermined outlet or branch channel of the substrate. The tag signal may be collected by a microscope and measured by a detector such as a photomultiplier tube (PMT). A computer is preferably used to digitize the PMT signal and to control the flow through methods such as those based on valve action. Alternatively, the signal can be recorded or quantified as a measure of the tag and/or its corresponding characteristic or marker, e.g., for the purpose of evaluation and without necessarily</p>

³³ '091 patent at 31:54-32:2; '193 patent at 31:16-31; '407 patent at 31:56-32:4; '573 patent at 32:26-41; '148 patent at 31:16-31; '083 patent at 30:62-31:10.

Quake PCT at 32:26-33:4	Ismagilov patents³³
necessarily proceeding to sort the molecules or cells.	proceeding to, for example, sort the plugs.

55. Another example of the close correspondence between Quake PCT and the '927 application is as follows:

Quake PCT at 37:29-38:5	'927 application at 22:29-23:4
A sample solution containing a mixture or population of molecule, cells or virions in a suitable carrier fluid (such as a liquid or buffer described above) is supplied to the sample inlet region, and droplets of the sample solution are introduced, at the droplet extrusion region, into the flow passing through the main channel. The force and direction of flow can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels). This permits the movement of the cells into one or more desired branch channels or outlet regions.	A sample solution containing a mixture or population of molecule, particles, or substance in a suitable carrier fluid (such as a liquid or buffer described above) is supplied to the sample inlet port, and plugs of the sample solution are introduced, at the plug-forming region, into the flow passing through the first channel. The force and direction of flow can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro osmotic flow (e.g., produced by electrodes at inlet and outlet channels). This permits the movement of the molecules, particles, or substances into one or more desired branch channels or outlet ports.

56. Much of this language was incorporated into and appears again in the issued Ismagilov patents:

Quake PCT at 37:29-38:5	Ismagilov patents³⁴
A sample solution containing a mixture or population of molecule, cells or virions in a suitable carrier fluid (such as a liquid or buffer described above) is supplied to the sample inlet region, and droplets of the sample solution are introduced, at the droplet extrusion region, into the flow passing through	The plug-fluids and carrier-fluids may be introduced through one or more inlets. . . . In the invention, plugs are formed by introducing the plug-fluid, at the plug-forming region, into the flow of carrier-fluid passing through the first channel. The force and direction of flow can be controlled by any desired method for

³⁴ '091 patent at 21:40-41, 21:48-54; '193 patent at 21:25-26, 33-39; '407 patent at 22:1-2, 9-15; '573 patent at 22:34-45, 22:42-48; '148 patent at 21:25-26, 33-39; '083 patent at 21:7-8, 15-21.

Quake PCT at 37:29-38:5	Ismagilov patents³⁴
the main channel. The force and direction of flow can be controlled by any desired method for controlling flow, for example, by a pressure differential, by valve action or by electro-osmotic flow (e.g., produced by electrodes at inlet and outlet channels). This permits the movement of the cells into one or more desired branch channels or outlet regions.	controlling flow, for example, by a pressure differential, or by valve action. This permits the movement of the plugs into one or more desired branch channels or outlet ports.

57. Another example of the close correspondence between Quake PCT and the '927 application is as follows:

Quake PCT at 34:32-35:7	'927 application at 22:12-20
The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets. Periodicity and droplet volume may also depend on channel diameter, the viscosity of the fluids, and shear pressure.	The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug-forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs. Periodicity and plug volume may also depend on channel diameter the viscosity of the fluids, and shear pressure.

58. Much of this language was incorporated into and appears again in the issued Ismagilov patents:

Quake PCT at 34:32-35:7	Ismagilov patents³⁵
--------------------------------	---------------------------------------

³⁵ '091 patent at 22:5-16; '193 patent at 21:56-67; '407 patent at 22:32-44; '573 patent at 22:65-23:9; '148 patent at 21:56-67; '083 patent at 21:38-49.

Quake PCT at 34:32-35:7	Ismagilov patents ³⁵
<p>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets. Periodicity and droplet volume may also depend on channel diameter, the viscosity of the fluids, and shear pressure.</p>	<p>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug-forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs. Periodicity and plug volume may also depend on channel diameter and/or the viscosity of the fluids.</p>

B. Quake and Quake '503

59. A patent application that ultimately issued as U.S. Patent No. 7,294,503 (“Quake ’503”) was filed on September 14, 2001 and assigned serial number 09/953,103. Like Quake PCT itself, this application named Stephen R. Quake and Todd Thorsen as inventors. The specification submitted with application 09/953,103 was published on May 16, 2002 as a part of U.S. Patent Publication No. 2002/0058332 A1 (“Quake”).³⁶

60. The specifications of Quake and Quake ’503 are essentially identical to that of Quake PCT.³⁷ In particular, all of the text quoted from Quake PCT in previous sections of this report is also present in Quake and Quake ’503.

³⁶ Quake and Quake ’503 are attached to my report as Exhibits E and F, respectively.

³⁷ There are minor differences in typography and formatting between the three publications. For example, certain words (*e.g.*, “Serial,” the names of months, and the names of states) that are fully written out in Quake PCT are abbreviated in Quake and Quake ’503, certain words (*e.g.*, “nonexistent”) that are not hyphenated in Quake PCT contain hyphens in Quake and Quake ’503 (*e.g.*, “non-existent”), and the paragraphs in Quake contain paragraph numbers.

C. Prosecution Histories of the Ismagilov patents

1. The '091 patent

61. The application that ultimately issued as the '091 patent was filed on May 9, 2003 and assigned serial number 10/434,970 (the "'970 application"). The '970 application named Rustem F. Ismagilov, Joshua David Tice, and Helen Song as inventors, and claimed priority back to the '927 provisional application.³⁸ The specification initially submitted with the '970 application contains all of the text cited as present in the '091 patent in § III.A above, and consequently exhibits the same similarities with Quake.

62. On March 10, 2005, the PTO mailed an Office Action that rejected numerous pending claims in the '970 application as anticipated by Quake.³⁹ The examiner stated:

Quake discloses "microfabricated crossflow devices and methods" (Title), including a method of conducting a reaction within at least one plug in a micro fluidic device, comprising introducing a carrier fluid (oil) and two different plug-fluids (substrate and enzyme) immiscible with the carrier fluid in the microchannel and applying pressure to the channel to form at least one plug that comprises a mixture of the plug-fluids, with the cross-section of the plug substantially of the size of a cross-section of the channel (page 1 [0003], Figures 22, 2A and B). The plug-fluids may contain surfactants, with the surfactants being fluorinated oils (page 3 [0017] and [0022]). "The interaction of the first and second biological materials [containing in the first and second plug-fluids] may produce a signal that can be detected, e.g., as the droplet passes through a detection region associated with the device" (page 3, [0018]). "A variety of channels for sample flow and mixing can be microfabricated on a single chip and can be positioned at any

Also, a reference to a "co-pending" provisional application in Quake PCT and Quake becomes a reference to a "now abandoned" provisional application in the more recent publication Quake '503.

³⁸ The '091 patent and its file history are attached to my report as Exhibits H and I, respectively.

³⁹ While Quake PCT was disclosed by the applicants in an IDS, Quake was not. But Quake was cited by the examiner as indicated by the (*) on the first page of the '091 patent.

location on the chip as the detection and discrimination or sorting points, e.g., for kinetic studies (10, 11) (page 9 [0079]).”

Ex. I at RDTX00000912.

63. On June 10, 2005, a response was filed by the applicants. Various claims were amended and arguments in favor of patentability were included in the response, including the following:

As noted by the examiner, Quake teaches that his system can be used to combine enzymes and substrates as discussed in Example 13 and illustrated in Figure 22. However, Quake does not use the method of the invention to combine the substrate and enzyme. Instead . . . Quake requires at least three steps before the reactants mix (1) introducing drops of the enzyme into a first conduit, (2) introducing drops of substrate into the first conduit and (3) timing the introduction of the substrate drops to allow them to merge with the enzyme drops. (See for example, Example 13 ; “By timing release of the these [substrate] droplets to occur as a droplet of enzyme passes through the second droplet extrusion region, the two droplets are combined and the enzyme is able to react with the substrate.” ¶ 316). Figure 22 reinforces that Quake first forms an enzyme drop in the carrier fluid BEFORE combining that drop with a substrate drop.

Ex. I at RDTX00000927.

64. Although the applicants distinguished Quake on the basis of features allegedly present in Quake’s Figure 22, significant portions of Quake’s own description of its Figure 22 appear nearly verbatim in the ’927 application, and both the specification initially filed with the ’970 application and the specification of the ’091 patent refer to this description as an “embodiment”:

Quake PCT at 85:14-25	’927 application at 32:15-25	’970 application at 22:3-14, ’091 patent at 15:23-40
<i>An exemplary embodiment of such a device is illustrated in FIG.22. The device comprises</i> a main channel 2201 through which a	The device may comprise a first channel through which a pressurized stream or flow of	<i>In another embodiment, a substrate according to the invention may comprise</i> a first channel through which a pressurized stream or flow of

Quake PCT at 85:14-25	'927 application at 32:15-25	'970 application at 22:3-14, '091 patent at 15:23-40
<p>pressurized stream or flow of a first fluid (e.g., oil) is passed, and two or more inlet channels 2202 and 2203 which intersect the main channel at droplet extrusion regions 2204 and 2205, respectively.</p> <p>Preferably, these inlet channels are parallel to each other and each intercept the main channel at a right angle. In specific embodiments wherein the droplets introduced through the different extrusion regions are mixed, the inlet channels are preferably close together along the main channel. For example, the main channel will typically have a diameter of 60 μm, that tapers to 30 μm at or near the droplet extrusion regions. The inlet channels also preferably have a diameter of about 30 μm and, in embodiments where droplet mixing is preferred, are separated by a distance along the main channel equal to approximately the diameter of the inlet channel (i.e., about 30 μm).</p>	<p>a first fluid (e.g., oil) is passed, and two or more inlet channels which intersect the first channel at plug-forming regions, respectively.</p> <p>Preferably, these inlet channels are parallel to each other and each intercept the first channel at a right angle. In specific embodiments wherein the plugs introduced through the different plug forming regions are mixed, the inlet channels are preferably close together along the first channel. For example, the first channel will typically have a diameter of 60 μm, that tapers to 30 μm at or near the plug-forming regions. The inlet channels also preferably have a diameter of about 30 μm and, in embodiments where plug mixing is preferred, are separated by a distance along the first channel equal to approximately the diameter of the inlet channel (i.e., about 30 μm).</p>	<p>a carrier-fluid is passed, and two or more inlet channels which intersect the first channel at plug-forming regions and through which a pressurized stream or flow of plug fluids pass.</p> <p>Preferably, these inlet channels are parallel to each other and each intercept the first channel at a right angle. In specific embodiments wherein the plugs introduced through the different plug forming regions are mixed, the inlet channels are preferably close together along the first channel. For example, the first channel may have a diameter of 60 μm that tapers to 30 μm at or near the plug-forming regions. The inlet channels then also preferably have a diameter of about 30 μm and, in embodiments where plug mixing is preferred, are separated by a distance along the first channel approximately equal to the diameter of the inlet channel (i.e., about 30 μm).</p>

65. On August 30, 2005, the PTO responded by mailing an examiner's amendment (authorized by the applicant during a phone interview) and an accompanying notice of allowability. The examiner's statement of reasons for allowance was as follows:

[T]he examiner agrees with the Applicants arguments that that the prior art teaching formation of drops with different reagents followed by mixing drops and performing reactions in drops is

different from the present invention teaching forming a plug (a drop) from two flows with different reagents; this is a principle difference in that while the prior art does not allow controlling or monitoring reaction kinetics by measuring exact time of the beginning of reaction, the present method allows that because this time is when the first plug is formed.

Ex. I at RDTX00000955.

66. I understand, for example from Dr. Chang's (one of 10X's technical experts in this matter) analysis, that the fact that applicants copied relevant portions from Quake would have been important to considering the patentability of the claims under examination. For example, relying in part on Quake's disclosures and description of Figure 22, Dr. Chang explains that multiple claims allowed by the examiner are in fact rendered obvious by Quake. This is the very same description that was copied by the applicants. While I do not offer a technical explanation of the prior art or claimed subject matter, the applicants' copying of the exact prior art under discussion would have been material to an examiner's consideration of the applicants' arguments regarding that same prior art. For example, under PTO procedures the examiner would likely have questioned the applicants regarding the copied passages to determine if the subject matter was part of the invention or part of the prior art and determine whether the applicants truly added anything novel or non-obvious beyond the copied material.⁴⁰ The copied

⁴⁰ See MPEP § 704.10 and 37 C.F.R. § 1.105. For example, Rule 1.105(a) provides examples of types of information that an examiner would seek from applicants in appropriate circumstances:

1.105(a)(1): In the course of examining or treating a matter in a pending or abandoned application, in a patent, or in a reexamination proceeding, including a reexamination proceeding ordered as a result of a supplemental examination proceeding, the examiner or other Office employee may require the submission, from individuals identified under § 1.56(c), or any assignee, of such information as may be reasonably necessary to properly examine or treat the matter, for example:

portions of the applicants' specification could also inform the broadest reasonable scope of the claims under review. However, applicants did not disclose to the examiner their copying of Quake.

2. The '407 patent

67. The application that ultimately issued as the '407 patent was filed on February 9, 2011 and assigned serial number 13/024,145 (the "'145 application"). The '145 application named Rustem F. Ismagilov, Joshua David Tice, Cory John Gerdtz, and Bo Zheng as inventors,

(i) Commercial databases: The existence of any particularly relevant commercial database known to any of the inventors that could be searched for a particular aspect of the invention.

(ii) Search: Whether a search of the prior art was made, and if so, what was searched.

(iii) Related information: A copy of any non-patent literature, published application, or patent (U.S. or foreign), by any of the inventors, that relates to the claimed invention.

(iv) Information used to draft application: A copy of any non-patent literature, published application, or patent (U.S. or foreign) that was used to draft the application.

(v) Information used in invention process: A copy of any non-patent literature, published application, or patent (U.S. or foreign) that was used in the invention process, such as by designing around or providing a solution to accomplish an invention result.

(vi) Improvements: Where the claimed invention is an improvement, identification of what is being improved.

(vii) In Use: Identification of any use of the claimed invention known to any of the inventors at the time the application was filed notwithstanding the date of the use.

(viii) Technical information known to applicant. Technical information known to applicant concerning the related art, the disclosure, the claimed subject matter, other factual information pertinent to patentability, or concerning the accuracy of the examiner's stated interpretation of such items.

and claimed priority back to the '927 provisional application.⁴¹ The specification initially submitted with the '145 application contains all of the text cited as present in the '407 patent in § III.A above, and consequently exhibits the same similarities with Quake. Although the examiner never rejected any pending claims in the '145 application for anticipation or obviousness in view of Quake PCT or Quake, the examiner's notice of allowance of the claims in the '407 patent contained the following statement:

Quake and Thorsen in [Quake] (IDS) disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil; however, the droplets in the oil are not formed from two continuously flowing immiscible fluids, but rather by a discrete dispensing the solution with biological material into the carrier fluid. This is a different approach from the claimed invention.

Ex. K at RDTX00002975.

68. I understand, for example from Dr. Chang's analysis, that the fact that applicants copied relevant portions from Quake would have been important to considering the patentability of the claims under examination, especially in view of this particular reason for allowance. For example, Dr. Chang explains that the examiner's basis for allowing the claims to issue over Quake was technically incorrect because (among other reasons) Quake does in fact disclose droplet formation from two continuously flowing immiscible fluids. And Dr. Chang relies, in part, on passages in Quake that were copied by Ismagilov to evidence this fact. While I do not offer a technical explanation of the prior art or claimed subject matter, the applicants' copying of the exact prior art under discussion would have been relevant to an examiner's consideration of the reasons for allowance over that same prior art. For example, the examiner would likely have

⁴¹ The '407 patent and its file history are attached to my report as Exhibits J and K, respectively.

questioned the applicants regarding the copied passages to determine if it was part of the applicants' invention or the prior art and to determine whether the applicants truly added anything novel or non-obvious beyond the copied passages and related prior art disclosures.⁴² The copied portions of the applicants' specification could also inform the broadest reasonable scope of the claims under review. However, applicants did not disclose to the examiner their copying of Quake.

3. The '193 patent

69. The application that ultimately issued as the '193 patent was filed on February 9, 2011 and assigned serial number 13/024,155 (the "'155 application"). The '155 application named Rustem F. Ismagilov, Joshua David Tice, Cory John Gerdts, and Bo Zheng as inventors, and claimed priority back to the '927 provisional application.⁴³ The specification initially submitted with the '155 application contains all of the text cited as present in the '193 patent in § III.A above, and consequently exhibits the same similarities with Quake.

70. Although the examiner never rejected any pending claims in the '155 application for anticipation or obviousness in view of Quake PCT or Quake, the examiner's notice of allowance of the claims in the '193 patent contained the following statement:

Quake and Thorsen in [Quake] (IDS) disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil; however, the droplets in the oil are not formed from two continuously flowing immiscible fluids, but rather by a discrete dispensing the solution with biological material into the carrier fluid. This is a different approach from the claimed invention.

Ex. M at RDTX00002245-46.

⁴² See MPEP § 704.10 and 37 C.F.R. § 1.105.

⁴³ The '193 patent and its file history are attached to my report as Exhibits L and M, respectively.

71. I understand, for example from Dr. Chang's analysis, that the fact that applicants copied relevant portions from Quake would have been important to considering the patentability of the claims under examination, especially in view of this particular reason for allowance. For example, Dr. Chang explains that the examiner's basis for allowing the claims to issue over Quake was technically incorrect because (among other reasons) Quake does, in fact, disclose droplet formation from two continuously flowing immiscible fluids. And Dr. Chang relies, in part, on passages in Quake that were copied by Ismagilov to evidence this fact. While I do not offer a technical explanation of the prior art or claimed subject matter, the applicants' copying of the exact prior art under discussion would have been relevant to an examiner's consideration of the reasons for allowance over that same prior art. For example, the examiner would likely have questioned the applicants regarding the copied passages to determine if it was part of the applicants' invention or the prior art and to determine whether the applicants truly added anything novel or non-obvious beyond the copied passages and related prior art disclosures.⁴⁴ The copied portions of the applicants' specification could also inform the broadest reasonable scope of the claims under review. However, applicants did not disclose to the examiner their copying of Quake.

4. The '573 patent

72. The application that ultimately issued as the '573 patent was filed on February 9, 2011 and assigned serial number 13/024,171 ("the '171 application"). The '171 application named Rustem F. Ismagilov, Joshua David Tice, Cory John Gerdts, and Bo Zheng as inventors,

⁴⁴ See MPEP 704.10 and 37 CFR 1.105.

and claimed priority back to the '927 provisional application.⁴⁵ The specification initially submitted with the '171 application contains all of the text cited as present in the '573 patent in § III.A above, and consequently exhibits the same similarities with Quake.

73. Although the examiner never rejected any pending claims in the '171 application for anticipation or obviousness in view of Quake PCT or Quake, the examiner's notice of allowance of the claims in the '573 patent contained the following statement:

Quake and Thorsen in [Quake] (IDS) disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil; however, the droplets in the oil are not formed from two continuously flowing immiscible fluids, but rather by a discrete dispensing the solution with biological material into the carrier fluid. This is a different approach from the claimed invention. Also, the separated droplets are not collected in a tube; does not teach or fairly suggest a method of collecting the plugs separated by an immiscible carrier fluid in a sample tube, the plugs comprising at least one biological molecule and optionally at least one reagent for conducting a reaction with it, and created by the recited steps.

Ex. O at RDTX00001605-06.

74. The only appearance of the phrase "sample tube" in the specification of the '573 patent occurs in a paragraph that is identical to a paragraph in the '927 application, and to a paragraph in Quake PCT:

Quake PCT at 27:9-32	'927 application at 27:21-23	'573 patent at 18:27-30
The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using	The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using	The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using

⁴⁵ The '573 patent and its file history are attached to my report as Exhibits N and O, respectively.

Quake PCT at 27:9-32	'927 application at 27:21-23	'573 patent at 18:27-30
micropipettes.	micropipettes.	micropipettes.

75. As shown in the table above, the applicants' sole discussion of the "sample tube" in their patent specification was copied verbatim from the Quake prior art reference. This is important because the examiner's notice of allowance specifically references a purported failure of the prior art to teach the collection of droplets in a tube, without full knowledge that the applicants actually copied the written description of this feature from that exact prior art that the examiner stated does not teach the claimed invention.

76. I further understand, for example from Dr. Chang's analysis, that the fact that applicants copied relevant portions from Quake would have been important to considering the patentability of the claims under examination, especially in view of this particular reason for allowance. For example, Dr. Chang explains that the examiner's basis for allowing the claims to issue over Quake was technically incorrect because (among other reasons) Quake does, in fact, disclose droplet formation from two continuously flowing immiscible fluids. And Dr. Chang relies, in part, on passages in Quake that were copied by Ismagilov to evidence this fact. While I do not offer a technical explanation of the prior art or claimed subject matter, the applicants' copying of the exact prior art under discussion would have been relevant to an examiner's consideration of reasons for allowance over that same prior art. For example, the examiner would have questioned the applicants regarding the copied passages to determine if it was part of the applicants' invention or the prior art and to determine whether the applicants truly added anything novel or non-obvious beyond the copied passages and related prior art disclosures.⁴⁶ The copied portions of the applicants' specification could also inform the broadest reasonable

⁴⁶ See MPEP 704.10 and 37 CFR 1.105.

scope of the claims under review. However, applicants did not disclose to the Examiner their copying of Quake.

77. On May 6, 2015, 10X filed a petition for *inter partes* review (“IPR”) of the ’573 patent challenging the validity of the claims on several grounds, including on the ground that multiple claims in the ’573 patent were anticipated by Quake. 10X’s petition for *inter partes* review included a chart comparing the language of claim 1 of the ’573 patent to disclosures in Quake. All of the disclosures cited in this chart are also present in Quake PCT. With the citations to Quake changed to the corresponding citations to Quake PCT, 10X’s chart is as follows:

Ismagilov ’573 patent claim 1	Quake PCT
A method for obtaining a collection of plugs comprising at least one biological molecule and, optionally, at least one or more chemical reactants for conducting a reaction with the biological molecule, the method comprising the steps of:	“A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region.” Abstract.
providing a microfluidic system comprising at least a first and second channel, the first and second channels configured to intersect with each other at a junction,	<p>“A microfluidic device provided by the invention comprises a main channel and at least one inlet region which is in communication with the main channel at a droplet extrusion region.” 6:4-6.</p> <p>“The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel, e.g., at an angle that is perpendicular to the stream of oil.” 1:18-24.</p>

Ismagilov '573 patent claim 1	Quake PCT
	<p>“The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used.” 79:23-27.</p>
<p>and an outlet adapted for receiving a segment of tubing or a sample tube;</p>	<p>“A group of manifolds (a region consisting of several channels which lead to or from a common channel) can be included to facilitate movement of the cell sample from the different analysis units, through the plurality of branch channels and to the appropriate solution outlet. Manifolds are preferably microfabricated into the chip at different levels of depth. Thus, devices of the invention having a plurality of analysis units can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube.” 44:12-20.</p> <p>“A ‘discrimination region’ or ‘branch point’ is a junction of a channel where the flow of molecules, cells or virions can change direction to enter one or more other channels, e.g., a branch channel, depending on a signal received in connection with an examination in the detection region. . . . Alternatively, a branch channel may also have an outlet region and/or terminate with a well or reservoir to allow collection or disposal of the molecules, cells or virions.” 20:1-17.</p>
<p>continuously flowing an aqueous fluid containing the at least one biological molecule and, optionally, the at least one or more reagents for conducting the reaction with the biological molecule, through the first channel;</p>	<p>“The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” 1:18-21.</p>

Ismagilov '573 patent claim 1	Quake PCT
	<p data-bbox="829 268 1437 443">“Fluid flows continuously through the system and there is no need for charged droplets, so that many difficult technical issues associated with traditional, e.g., F ACS devices are avoided.” 5:21-23.</p> <p data-bbox="829 485 1437 806">“[A] first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region. The sample fluid is, specifically, a fluid which is incompatible with the extrusion fluid and contains the biological material or sample.” 7:30-8:2.</p> <p data-bbox="829 848 1437 1604">“Thus, for example, in preferred embodiments wherein droplets of aqueous solution are extruded into a pressurized stream of oil, the pressures of the oil and/or fluid lines are adjusted so that the pressure difference of the oil and water channels at the droplet extrusion region is zero, and the oil and water are in a state of equilibrium. This can be visually observed. Droplet extrusion can then be initiated by slightly adjusting the pressure difference between the different fluids (i.e., at the different inlet lines) so that the droplet fluid (e.g., water) enters the main channel and is sheared off at a fixed frequency. A preferred frequency is 1 Hz because the frequency 15 with which droplets are sheared off into the main channel depends on the pressure difference between the different fluids, the frequency can be readily adjusted by simply adjusting the pressures of the individual fluid lines.” 79:7-17.</p> <p data-bbox="829 1646 1437 1890">“A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the</p>

Ismagilov '573 patent claim 1	Quake PCT
	biological material are deposited into the main channel through the droplet extrusion Region.” Abstract.
continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel;	<p>“The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” 1:18-21.</p> <p>“[A] first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes or flows through the inlet region.” 7:30-8:1.</p> <p>“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.” 34:17-21.</p> <p>“The flow stream in the main channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed.” 34:23-24.</p> <p>“Fluid flows continuously through the system and there is no need for charged droplets, so that many difficult technical issues associated with traditional, e. g., FACS devices are avoided.” 5:21-23.</p>
forming a plurality of the plugs of the aqueous fluid containing the at least one biological molecule and, optionally, the at least one or more reagents for conducting the reaction with the biological molecule, by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the first and	“Preferably, the fluid introduced to the main channel through the extrusion region is “incompatible” (i.e., immiscible) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel.” 19:29-32.

Ismagilov '573 patent claim 1	Quake PCT
second channels,	<p data-bbox="829 268 1437 552">“In preferred embodiments, the droplet extrusion region comprises a T-shaped junction between the inlet region and the main channel, so that the second fluid enters the main channel at an angle perpendicular to the flow of the first fluid, and is sheared off into the flow of the first fluid in the main channel.” 6:23-27.</p> <p data-bbox="829 590 1437 951">“A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region.” Abstract.</p> <p data-bbox="829 989 1437 1755">“For particles (e.g., cells, including virions) or molecules that are in droplets (i.e., deposited by the droplet extrusion region) within the flow of the main channel, the channels of the device are preferably rounded, with a diameter between about 2 and 100 microns, preferably about 60 microns, and more preferably about 30 microns at the crossflow area or droplet extrusion region. This geometry facilitates an orderly flow of droplets in the channels. See e.g. FIG. 16B. Similarly, the volume of the detection region in an analysis device is typically in the range of between about 10 femtoliters (fl) and 5000 fl, preferably about 40 or 50 fl to about 1000 or 2000 fl, most preferably on the order of about 200 fl. In preferred embodiments, the channels of the device, and particularly the channels of the inlet connecting to a droplet extrusion region, are between about 2 and 50 microns, most preferably about 30 microns.” 26:30-27:8.</p> <p data-bbox="829 1793 1437 1890">“In one preferred embodiment, droplets at these dimensions tend to conform to the size and shape of the channels, while maintaining</p>

Ismagilov '573 patent claim 1	Quake PCT
	<p>their respective volumes. Thus, as droplets move from a wider channel to a narrower channel they become longer and thinner, and vice versa." 27:9-13.</p>
<p>the plugs being substantially surrounded by the immiscible carrier fluid flowing through the channel,</p>	<p>"In embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous droplets are encapsulated or separated from each other by oil." 29:32-30:2.</p>
<p>wherein at least one plug of the plurality comprises the at least one biological molecule and, optionally, the at least one or more reagents for conducting the reaction with the biological molecule; and collecting the plurality of the plugs in the segment of tubing or the sample tube,</p>	<p>"The concentration (i.e., number) of molecules, cells or virions in a droplet can 30 influence sorting efficiently and therefore is preferably optimized. In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions. This is to ensure that for the large majority of measurements, the level of reporter measured in each droplet as it passes through the detection region corresponds to a single molecule, cell or virion and not to two or more molecules, cells or virions." 36:29 - 37:4.</p> <p>"An 'outlet region' is an area of a micro fabricated chip that collects or dispenses molecules, cells or virions after detection, measurement or sorting." 18:26-27.</p> <p>"Thus, devices of the invention having a plurality of analysis units can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml 20 centrifuge tube. Collection can also</p>

Ismagilov '573 patent claim 1	Quake PCT
	be done using micropipettes.” 44:16-20.
wherein the plugs remain separated by the carrier immiscible fluid.	“In embodiments of the invention where an aqueous phase is combined with an oil phase, aqueous droplets are encapsulated or separated from each other by oil.” 29:32-30:2.

78. The Patent Trial and Appeal Board (the “PTAB”) elected to institute trial on all claims of the '573 patent, including on the ground that various claims were anticipated by Quake. Shortly after the decision to institute trial, the owner of the '573 patent requested adverse judgment in the proceeding, requesting that all claims of the '573 patent be cancelled. The PTAB granted the request for adverse judgment and all claims of the '573 patent were correspondingly canceled by the PTAB in a final decision dated February 3, 2016. In other words, the PTAB determined from the evidence that the examiner’s original determination that the claims in the '573 patent were patentable over Quake was likely incorrect, and the patent owner did not argue to the contrary.

5. The '083 patent

79. The application that ultimately issued as the '083 patent was filed on October 30, 2006 and assigned serial number 11/589,700 (“the '700 application”). The '700 application named Rustem F. Ismagilov, Joshua David Tice, Helen Song, and Lewis Spencer Roach, Jr. as inventors, and claimed priority back to the '927 provisional application.⁴⁷ The specification

⁴⁷ The '083 patent and its file history are attached to my report as Exhibits R and S, respectively.

initially submitted with the '700 application contains all of the text cited as present in the '083 patent in § III.A above, and consequently exhibits the same similarities with Quake.

80. This application was examined by a different patent examiner than the other applications discussed above. On March 30, 2010, the PTO mailed an Office Action that rejected numerous pending claims in the '700 application as anticipated by Quake '503. Ex. O at 10X-000253349–55.

81. For example, rejected claim 41 claimed:

A method of conducting a reaction within at least one plug comprising the steps of:

introducing a carrier-fluid into a first microchannel of a device,

introducing at least one stream of plug-fluid into a first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid; wherein:

the at least one plug-fluid comprises water and at least one reagent for an autocatalytic reaction; the at least one plug-fluid is immiscible with the carrier-fluid; and each plug is substantially surrounded by carrier-fluid.

Ex. S at 10X-00253334.

82. The examiner's rejection stated, *inter alia*,

Quake teaches a microfluidic device and method for sorting biological materials. The device is best shown in Figures 4A-6, 8, 14, 15 and 22 and described in general in columns 3-6. The device includes a microchannel, carrier fluid, and an aqueous fluid plug surrounded by the carrier fluid. The carrier fluid may include polyethylene glycol (column 19, lines 18-60) and fluorinated oils (column 24, lines 18-38).]).

Ex. S at 10X-000253354.

83. In a response dated June 29, 2010, the applicants traversed this rejection, making several arguments in favor of patentability, including the following:

The Examiner asserted that Quake ['503] discloses fluorinated oils, referring to column 24, lines 18-38 (Office Action, page 4). Quake ['503], however, expressly teaches that the fluorinated oil is a surfactant or an additive or agent, which is used to coat the channel (see column 24, lines 18-21 and 32-34).

Ex. S at 10X-00253383.

84. The applicants did not mention that some of the material cited in Quake '503 to distinguish Quake '503, specifically Quake '503 at 24:18-21, had been copied by the applicants in both the '927 application and the specification of what became the '083 patent:

Quake '503 at 24:18-21	'927 application at 12:8-21, 22:21-28	'083 patent at 20:13-15, 20:29-31
The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.	The fluids used in the invention may contain additives, such as agents which reduce surface tensions (<i>e.g.</i> , surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water.	The carrier-fluid or plug-fluid, or both may contain additives, such as agents that reduce surface tensions (<i>e.g.</i> , surfactants). Exemplary surfactants include Tween™, Span™, and fluorinated surfactants (such as Zonyl™ (Dupont, Wilmington Del.))

85. The applicants never disclosed to the Examiner that they had copied these and other portions of Quake '503 into their own patent application.

86. Subsequent rejections in view of Quake '503 occurred on September 15, 2010 and October 5, 2011. Ex. S at 10X-000253868, 10X-000253979, and none of these rejections were ever disclosed to the Examiner of applications Nos. 13/024,145 (which issued as the '407 patent) and 13/024,155 (which issued as the '193 patent), which were pending as of October 5, 2011. Neither these rejections nor a subsequent rejection in view of Quake '503, Ex. S at 10X-000254400, were disclosed to the Examiner of the later-filed application No. 13/563,327 (which claimed priority to the '927 application and issued as the '148 patent).

IV. ANALYSIS

87. Based on my experience, it is unusual and improper for a patent applicant to copy substantial portions of another inventor's patent application into his application without attribution to the other inventor. In this case, the Ismagilov patents copy substantial portions of the Quake PCT without attributing those portions of the application to Quake. Further, those copied portions appear in the section of the patents entitled "Detailed Description According to The Invention." While it sometimes may be appropriate to explain or even incorporate information from another inventor in the "Background" section of a patent application to describe the state of the art, it would be improper to include material from another's patent application *in the detailed description of the applicant's invention* without explaining to the PTO that it was the invention of another.⁴⁸ Generally, subject matter contained in the Detailed Description of the Invention section of a patent application is assumed to be the invention of the applicant unless otherwise stated. In my experience, an examiner would assume that information listed by an applicant as a "detailed description of the invention" or as a specific "embodiment" of the invention, reflects the work of the applicant and not work copied from the prior art. In patent parlance, an "embodiment" of an invention is a specific example that the applicant believes includes the features of his invention. Examiners look to an applicant's identified "embodiments" to understand what it is that the applicant believes he invented, the broadest reasonable scope that should be afforded the applicant's claims, and how such inventions may be different from the prior art.⁴⁹

⁴⁸ See 37 C.F.R. § 1.71(b).

⁴⁹ See 37 C.F.R. § 1.71 (b) ("The specification must set forth the precise invention for which a patent is solicited, in such manner as to distinguish it from other inventions and from what is old.").

88. In my opinion, the applicants' conduct with regard to the copying of Quake was a breach of the applicants' duty of candor and good faith. This conduct includes, for example, copying the Quake reference and identifying it as an embodiment of the applicants own invention, failing to disclose to the examiner that significant portions of the specification were copied from the prior art, failing to explain to the examiner that certain passages related to rejections issued by the examiner were copied from the prior art. Additionally, in a few sections of the applicants' specification, applicant expressly identifies copied passages as "the invention," which further affirmatively misleads the examiner that the Quake prior art is the applicants' own work. Moreover, the applicants' failure to disclose rejections, over the very same prior art and issued by a different examiner, in related applications is also a breach.⁵⁰ Further, I understand from Dr. Chang that the examiner was mistaken in his understanding of the Quake prior art, therefore the applicants should have corrected the examiner, based on their own knowledge of the prior art (as evidenced by the substantial copying they did). These activities, each alone or in combination, represent breaches of the applicants' duty of candor and good faith.⁵¹

⁵⁰ See, e.g., MPEP § 704.11 (information is reasonably necessary to examination "where the application file, or other related applications or publications authored by the applicant, suggests the applicant likely has access to information necessary to a more complete understanding of the invention and its context. In this situation, the record suggests that the details of such information may be relevant to the issue of patentability, and thus shows the need or information in addition to that already submitted by the applicant."); *Larson Mfg. Co. of South Dakota, Inc. v. Aluminart Products Ltd.*, 559 F.3d 1317 (Fed. Cir. 2009) ("Because the [related] Office Actions contained another examiner's adverse decisions about substantially similar claims, and because the [related] Office Actions are not ... the district court correctly found the withheld Office Actions material.")

⁵¹ 37 C.F.R. § 1.56. See also, e.g., MPEP § 2001.04 ("The rules as adopted serve to remind individuals associated with the preparation and prosecution of patent applications of their duty of candor and good faith in their dealings with the Office, and will aid the Office in receiving, in a timely manner, the information it needs to carry out effective and efficient examination of patent applications.")

89. Here, applicants expressly described passages of their patents directly copied from the Quake PCT as “embodiments” of their invention when providing their “detailed description of the invention.” In addition to being unusual and improper, this copying was not disclosed to the examiner. As described above, the examiner considered Quake important to the patentability of the subject claims, for example by issuing rejections over Quake and specifically distinguishing Quake in the notice of allowance. The copied portions of the applicants’ specification could also inform the broadest reasonable scope of the claims under review. There is no indication that the applicant ever made the examiner aware of its substantial copying. Had the examiner been aware of this copying, the MPEP provides that the examiner should have taken some additional actions in considering the patentability of the claims, such as following the MPEP provisions related to requirements for information.⁵² Additionally the examiner would have considered whether the claimed subject matter was derived from another and whether to issue an appropriate rejection under 35 U.S.C. § 102(f) and/or consider whether to invoke interference proceedings.

90. I further understand that the technical content of the disclosures is substantially the same, as between Quake and the claims under examination in at least the ’573 patent. Dr. Chang has stated that claims in each of the other Ismagilov patents are anticipated by Quake and/or rendered obvious by Quake (alone or in combination with other references). I further understand that, according to Dr. Chang, the examiner made technical errors in its consideration of Quake. Had the examiner been made aware of the applicants’ copying, the examiner would have likely questioned the applicants regarding the copied passages to determine if it was part of

⁵² See MPEP § 704.10 and 37 C.F.R. § 1.105. See also, e.g., MPEP § 2304 (“**The suggestion for an interference may come from an applicant or from an examiner.**”); 37 C.F.R. § 41.202.

the applicants' invention or the prior art.⁵³ Dr. Chang explains that the claims are anticipated by Quake or rendered obvious by Quake (alone or in combination with other references). In view of this, they would not have been allowed to issue if the proper teaching of Quake were considered by the examiner.

91. Further, it is improper to attempt to claim subject matter derived from another. Under PTO policy, if the examiner became aware of such derivation, the examiner would reject such claims under 35 U.S.C. §102 (f).⁵⁴ The applicant would then be required to argue against this rejection and/or amend its claims or specification. At least because the applicants never disclosed to the examiner the extent of its copying of the prior art, the examiner was not able to fully explore this and potential other issues (such as procedures related to interferences) during prosecution.

92. If indeed, any subject matter in an application is not that of the applicant, but is derived from another, the PTO would expect the applicant to disclose such a fact. Additionally, the PTO would further presume, because applicants are under an ongoing duty of candor and good faith, that there are no such material facts that need to be disclosed if the applicant does not disclose anything. If the applicant does disclose knowledge or use of specific prior art, an examiner will use the applicant's admission of prior knowledge of another as "applicant's

⁵³ See MPEP § 704.10 and 37 C.F.R. § 1.105.

⁵⁴ See MPEP § 2137. See also, e.g., 37 C.F.R. § 1.106(d) ("Subject matter which is developed by another person which qualifies as prior art only under 35 U.S.C. § 102(f) or (g) may be used as prior art under 35 U.S.C. § 103."); *OddzOn Prod., Inc. v. Just Toys, Inc.*, 122 F.3d 1396, 1403–04 (Fed. Cir. 1997) ("We therefore hold that subject matter derived from another not only is itself unpatentable to the party who derived it under § 102(f), but, when combined with other prior art, may make a resulting obvious invention unpatentable to that party under a combination of §§ 102(f) and 103.")

admitted prior art” when determining patentability.⁵⁵ That is, often an applicant will disclose to the examiner what is known or admitted to be in the prior art when describing his or her invention. The examiner may then use that admission, when appropriate, for determining the patentability of the claims of the application. For example, an examiner may issue rejections based on “Admitted Prior Art” when considering the patentability of applications that include such admissions.⁵⁶ Here, the applicants did not disclose to the examiner that they copied portions of the Quake prior art into their application, and the examiner therefore did not have the opportunity to fully explore possible rejections in view of “Admitted Prior Art” during prosecution of the subject claims.

93. As another example, if the examiner had been informed of the applicants’ copying of Quake into the “detailed description” of the invention, the examiner should have issued objections to the specification and required the applicant to revise the specification to clarify what portions were attributable to Quake and what portions were the applicants’ own work.⁵⁷ That would have aided the examiner in determining whether anything reflecting the applicants’ own work, as opposed to what they copied from Quake, was novel and non-obvious over Quake. Once again, because the applicants did not disclose their copying to the examiner, the examiner did not fully consider this issue and take appropriate action during prosecution.

94. In addition, as noted above, during the prosecution of the ’091 patent, the examiner made a rejection based on Quake.⁵⁸ When rebutting the rejection, the applicants argued

⁵⁵ See MPEP § 2129.

⁵⁶ *Id.*

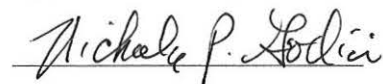
⁵⁷ See MPEP § 2137.

⁵⁸ While Quake PCT was disclosed by the applicants in an IDS, Quake was not and was cited by the examiner as indicated by the (*) on the first page of the ’091 patent.

that Figure 22 of Quake reinforced the alleged distinction between Quake and the claims of the '970 application. However, significant portions of Quake's description of Figure 22 was incorporated into the Ismagilov '970 application as another embodiment "according to the invention." In other words, on the one hand, the applicants argued that Quake's description of Figure 22 distinguished the teaching of Quake from the claims of the '970 application, but on the other hand, applicants incorporated Quake's description of Figure 22 as part of their own invention. If the examiner had known that the applicants had copied the prior art's description of this figure, this fact would have been important in the examiner's consideration of the applicants' argument.

95. Dr. Chang has also explained that there are technical reasons why the Examiner would have reached different conclusions with regard to Quake if the examiner had full knowledge of the applicable facts, including the applicants' copying and the portions of the disclosure attributable to Quake. As evidenced by Dr. Chang's analysis, if prosecution had been conducted on the full set of facts, and had proceeded according to the appropriate MPEP provisions, the examiner would not have reached the same patentability decisions in view of the technical deficiencies identified by Dr. Chang.

August 21, 2017



Nicholas P. Godici

EXHIBIT E

Walter, Derek

From: Haber, Benjamin <BHaber@irell.com>
Sent: Sunday, August 27, 2017 10:11 AM
To: Walter, Derek; Reines, Edward
Cc: bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; 'Rawnsley@RLF.com'; #10X/RainDance [Int]
Subject: RE: 10X/Bio-Rad - Production

Derek,

I was speaking with you on the call last week, not Dennis.

In response to your question, as an example, Mr. Godici's opinions are relevant to, among other things, the weight to be afforded to the examiner's allowance of the claims in view of evidence that the PTO had no opportunity to evaluate before granting the patent. Mr. Godici explains, among other things, that prosecution would have proceeded differently in full view of the evidence including, for example, the applicants' substantial copying of a key prior art reference and non-disclosure of this fact.

As another example, Mr. Godici, with deep expertise in patent prosecution, also explains the prosecution history in detail, including the extensive and undisclosed copying of a key prior art reference. This further supports the conclusion that the patents are invalid in light of that prior art reference.

Also, as I asked on our call, please identify to us any specific paragraphs of Mr. Godici's report that you believe are objectionable.

Regards,
Ben Haber

From: Walter, Derek [mailto:Derek.Walter@weil.com]
Sent: Thursday, August 24, 2017 1:33 PM
To: Courtney, Dennis; ~Reines, Edward; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com
Cc: RainDance 10X Service; #10X/RainDance [Int]; Rawnsley, Jason J. (Rawnsley@RLF.com)
Subject: RE: 10X/Bio-Rad - Production

Dennis,

A few things I neglected to ask in the confusion of trying to handle this call on the road. First, can you confirm you are not contending that Mr. Godici's report is relevant to inequitable conduct? Second, you state that Mr. Godici's report is relevant to invalidity. Can you let us know what invalidity issues you believe the report is relevant to?

Thanks,

Derek

From: Courtney, Dennis [mailto:dcourtney@irell.com]
Sent: Tuesday, August 22, 2017 2:11 PM
To: Walter, Derek; Reines, Edward; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com

Cc: RainDance 10X Service; #10X/RainDance [Int]; Rawnsley, Jason J. (Rawnsley@RLF.com)

Subject: RE: 10X/Bio-Rad - Production

Derek,

Mr. Godici's report is proper and 10X will not withdraw it. Among other things, Mr. Godici opines on the prosecution of the patents in suit and the impact various actions taken or not taken by the applicants had on the examination at the Patent Office. Mr. Godici's opinions are relevant to, among other things, invalidity.

Regards,

Dennis

From: Walter, Derek [<mailto:Derek.Walter@weil.com>]

Sent: Monday, August 21, 2017 8:24 PM

To: Courtney, Dennis; ~Reines, Edward; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com

Cc: RainDance 10X Service; #10X/RainDance [Int]; Rawnsley, Jason J. (Rawnsley@RLF.com)

Subject: RE: 10X/Bio-Rad - Production

Dennis:

Mr. Godici's report addresses an alleged breach of the duty of candor. This appears to be an improper attempt to suggest there was inequitable conduct in the prosecution of the patents-in-suit even though there is no inequitable conduct allegation in this case. Please confirm by close of business Wednesday that 10X will withdraw this report.

Thanks,

Derek

From: Courtney, Dennis [<mailto:dcourtney@irell.com>]

Sent: Monday, August 21, 2017 2:50 PM

To: Walter, Derek; Reines, Edward; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com

Cc: RainDance 10X Service; #10X/RainDance [Int]; Rawnsley, Jason J. (Rawnsley@RLF.com)

Subject: 10X/Bio-Rad - Production

Counsel:

Please see attached. Mr. Godici is available for a deposition on October 12, 2017.

The exhibits may be accessed from the FTP site below:

Site: [ftp.irell.com](ftp://ftp.irell.com)

Username: 10x_prod

Site password (case sensitive): kmpe5Bio

Folder: Godici_Exhs

Connection method: Instead of a web browser, you must use an FTP application that supports TLS encryption to upload and download files from our FTP site. We recommend using the FileZilla FTP client, which is available for download at <http://www.irell.com/ftp>. For assistance, please contact the Irell Technical Assistance Center at 1-844-551-0709 or email ITAC@irell.com. Please provide them with the FTP username you are using.

Thank you,

Dennis

===

Dennis Courtney
Irell & Manella LLP
840 Newport Center Dr., Ste. 400
Newport Beach, CA 92660
(949) 760-5238

PLEASE NOTE: This message, including any attachments, may include privileged, confidential and/or inside information. Any distribution or use of this communication by anyone other than the intended recipient(s) is strictly prohibited and may be unlawful. If you are not the intended recipient, please notify the sender by replying to this message and then delete it from your system. Thank you.

The information contained in this email message is intended only for use of the individual or entity named above. If the reader of this message is not the intended recipient, or the employee or agent responsible to deliver it to the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited. If you have received this communication in error, please immediately notify us by email, postmaster@weil.com, and destroy the original message. Thank you.

The information contained in this email message is intended only for use of the individual or entity named above. If the reader of this message is not the intended recipient, or the employee or agent responsible to deliver it to the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited. If you have received this communication in error, please immediately notify us by email, postmaster@weil.com, and destroy the original message. Thank you.

EXHIBIT F

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

BIO-RAD LABORATORIES, INC. and)	
THE UNIVERSITY OF CHICAGO,)	
)	
Plaintiffs and Counterclaim)	
Defendants,)	
)	
v.)	
)	C. A. No. 15-152-RGA
10X GENOMICS, INC.,)	
)	
Defendant and Counterclaim)	
Plaintiff.)	
)	

**10X GENOMICS, INC.’S SECOND SUPPLEMENTAL INITIAL
PARAGRAPH 4(d) DISCLOSURE**

Pursuant to the Court’s Scheduling Order (D.I. 165) and Paragraph 4(d) of the Default Standard for Discovery, Defendant and Counterclaim Plaintiff 10X Genomics, Inc. (“10X”) hereby provides the following Second Supplemental Initial Invalidity Contentions for U.S. Patent Nos. 7,129,091 (the “’091 patent”); 8,304,193 (the “’193 patent”); 8,329,407 (the “’407 patent”); 8,822,148 (the “’148 patent”); and 8,889,083 (the “’083 patent”) (collectively, the “Ismagilov patents”).

I. Preliminary Statement

10X provides its Contentions subject to the following objections and reservation of rights:

1. Discovery in this matter is ongoing, as is 10X’s investigation regarding the potential grounds of invalidity. Bio-Rad Laboratories, Inc. (“Bio-Rad”) and the University of Chicago (collectively, “Plaintiffs”) have not yet produced requested documents and things relating to the Ismagilov patents. 10X’s Paragraph 4(d) Disclosure is given without prejudice to

10X's right to supplement or amend as additional facts are ascertained, analyses are made, research is completed, contentions are made, and claims are construed. 10X is permitted to supplement these initial disclosures pursuant to section 4, note 3, of the Default Standard. 10X further reserves the right to modify, supplement, amend, or otherwise alter these disclosures as discovery progresses, as permitted by the Federal Rules of Civil Procedure, by the Default Standard, or by order of the Court. In particular, 10X reserves the right to modify, supplement, amend, or otherwise alter these disclosures following an opportunity for expert discovery. These disclosures do not encompass patent unenforceability, which may be alleged separately by 10X.

2. 10X has relied upon the Court's claim construction orders, the ordinary meaning of claim terms, definitions in the patents, and Plaintiffs' apparent claim construction positions from Plaintiffs' Initial Paragraph 4(c) Disclosures served on June 14, 2016, to the extent any such constructions can be discerned. 10X's reliance on Plaintiffs' claim constructions should not be taken to mean that 10X in any way agrees with Plaintiffs' apparent claim constructions or that 10X is precluded from propounding alternative claim constructions or requesting Plaintiffs' actual claim construction positions in the future. 10X expressly reserves the right to propose alternative constructions to those advocated by Plaintiffs. 10X's investigation is continuing and is likely to uncover additional art. 10X will supplement its disclosures at appropriate times in light of newly discovered art or changes in claim constructions.

3. The disclosure of a reference as anticipating of a claim includes a disclosure of the reference for obviousness purposes should any element of the claim be determined by the Court to be absent from the reference. Moreover, 10X is at the present time unaware of the extent, if any, to which Plaintiffs will contend that elements of the claims are not disclosed in references identified by 10X as anticipatory. To the extent that Plaintiffs make any such claim

with respect to any limitation, 10X reserves the right to identify other references which may explain the inherency of, or make obvious the addition of, the allegedly missing element.

4. References disclosed as rendering a claim obvious are representative and are not intended to be exhaustive. Other references disclosing the same or similar elements may be substituted for the cited references. Additional obviousness combinations of the references identified below are possible, and 10X reserves the right to use any such combination(s) in this litigation. Motivation to combine references can be inferred generally for all references within the fields of art of the Ismagilov patents. Furthermore, where references refer to or cite one another, motivation to combine may be specifically inferred whether or not called out in a claim chart. Lastly, 10X's identification of motivation to combine references should not be taken as an admission or a representation that 10X will not rely upon other tests for obviousness in view of *KSR Int'l. Co. v. Teleflex Inc.*, 550 U.S. 398 (2007). This would include showing any of the following: (1) that the combination of elements was obvious to try; (2) that the combination of elements according to known methods yielded predictable results; (3) that the substitution of one known element for another obtained predictable results; (4) that the application of a known technique to a known device, method, or product ready for improvement yielded predictable results; or (5) that known work in one field of endeavor prompted variations of such work for use in either the same field or a different one based on design incentives or other market forces because the variations are predictable to one of ordinary skill in the art.

5. The identification of prior art that anticipates and/or renders obvious a particular claim element in these contentions is not an admission that the claim element satisfies the requirements of 35 U.S.C. § 112.¹ Where 10X asserts that a claim is invalid under

¹ All statutory citations are to Pre-AIA versions unless otherwise indicated.

35 U.S.C. § 112 (such as because of a failure to particularly point out and distinctly claim the alleged invention, failure to provide written description support, and/or failure to enable one of ordinary skill in the art to make and use the claimed invention), 10X has nonetheless provided prior art that anticipates or renders obvious the claim on the assumption that Plaintiffs will contend that those claims are definite, supported by an adequate written description, and adequately enabled.

6. In addition to the prior art identified in these contentions, and any future supplement to these contentions, 10X may rely on relevant portions of the Ismagilov patents, the prosecution histories of the Ismagilov patents, all references listed in the References Cited portion of the Ismagilov patents, all references cited or documents filed in IPR Nos. 2015-01156, 2015-01157, 2015-01158, 2015-01162, 2015-01163, and 2015-01560, and fact and expert testimony about the prior art.

7. 10X objects to the disclosure of information that is protected by the attorney-client privilege, attorney work-product immunity, the common-interest privilege, or any other applicable privilege or immunity.

8. 10X reserves the right to amend and/or supplement these contentions as fact and expert discovery and claim construction proceed.

II. Prior Art

10X identifies at least the prior art references set forth in Appendix A, which alone or in combination, render the asserted claims of the Ismagilov Patents invalid under 35 U.S.C. §§ 102 and/or 103. 10X reserves the right to identify additional prior art references as discovery progresses.

III. Anticipation and Obviousness

1. Anticipation of the '407 Patent

As detailed in the claim charts attached as Appendices B-D, every asserted claim of the '407 patent is anticipated by Quake *et al.*, U.S. Patent Application Publication No. 2002/0058322; Shaw Stewart, WO84/02000; Shaw Stewart, GB 2,097,692; and/or Shaw Stewart, The Development and Applications of a New Liquid Handling Device, Doctoral Thesis, University of London and the Imperial College of Science and Technology (1998). The citations to the prior art corresponding to the claim limitations in Appendices B-D are exemplary and are not intended to be exhaustive. For the claim charts in Appendices B-D, 10X identifies disclosures in the prior art concerning the preamble without regard to whether the preamble is a claim limitation. Terms in the preamble may not be claim limitations.

2. Obviousness

10X contends that the prior art listed in Appendix A, whether alone or in combination with each other, render the asserted claims of the Ismagilov patents obvious under 35 U.S.C. § 103, as set forth in the claim charts attached as Appendices E-I. In particular, each prior art reference may be combined with (1) information known to persons skilled in the art at the time of the alleged invention; (2) any other prior art references; (3) any additional prior art to be identified by 10X; and (4) any additional prior art identified or to be identified in prosecution of the Ismagilov patents or their related patent applications. The citations to the prior art corresponding to the claim limitations in Appendices E-I are merely exemplary and are not intended to be exhaustive. For the claim charts in Appendices E-I, 10X identifies disclosures in the prior art concerning the preamble without regard to whether the preamble is a claim limitation. Terms in the preamble may not be claim limitations.

(i) The '091 Patent

The following combinations render the '091 patent obvious. These combinations are merely exemplary and are not intended to be exhaustive:

- Certain asserted claims are rendered obvious by Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination.
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or

in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Kenis or Weigl, or Kopf-Sill or Floyd or Johnson or Erbacher or Whitesides, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).

A person of ordinary skill in the art would have been motivated to combine the references listed in Appendix E, including because these references share common subject matter and approaches, discuss related products and technologies, and/or were developed during the same time period. Most of the references concern conducting reactions by using immiscible carrier fluid to partition aqueous fluid within microfluidic devices. For example, Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials.” Ex. 1 at Abstract; Ex. 2 at Abstract. Droplets are formed using sample fluid that is incompatible with extrusion fluid, within which reactions are conducted. Ex. 1 at 8:14-9:2; Ex. 2 at ¶ [0020]. Quake also describes conducting a specific reaction using reagents contained in a single aqueous fluid. For example, Quake describes that:

In another embodiment, cells may produce a reporter *in vivo* (*e.g.* a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (*e.g.* benzene or naphthalene) with the net result

that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate. Sorting can also be done based on a threshold or window concentration of reaction product, which in turn can be correlated with the level of fluorescence. A second reagent or coupling enzyme can be used to enhance fluorescence. *See*, Affholter and Arnold (34) and Joo et al. (35). Any mechanism of this kind, including any reporter or combinations of substrate, enzyme and product can be used for detection and sorting in a like manner, so long as there is at least one way to detect or measure the presence or degree of the reaction of interest.

Ex. 1 at 51:19-52:5; Ex. 2 at [0170]. Kenis pertains to microfluidic devices in which “it is possible to bring a wide variety of solutions in contact with one another” “by using ‘Y’ or ‘T’ junctions (or their extensions to multiple streams).” Ex. 37 at 83:3-6.

A person of ordinary skill in the art would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions as taught by Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man. A skilled artisan would have been strongly motivated to perform such reactions in such

microfluidic reactors because doing so have provided the substantial benefits known to be associated with microfluidic reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Ex. 18 at 565. As another example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Ex. 7 at 24. It was also well known that miniaturization of reactions to the microfluidic level provided the substantial advantage of making reactors highly portable, energy efficient, and automated. Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 48 at 1047. Moreover, it was generally known that portable reactors were useful to aid physicians in development of treatment of various conditions. *Id.* For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” *Id.* It was thus known that using the droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to perform reactions advantageously would have allowed reactions to be performed in point of care diagnostic applications. Skilled artisans would also have recognized that performing reaction in the microfluidic reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako,

Thorsen, or Seki may enhance the precision of the reaction relative to then-traditional approaches.

Moreover, a skilled artisan would have fully expected the combination to be successful. The prior art shows that many reactions had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. A skilled artisan would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man.

As further evidence that a skilled artisan would have expected to be successful in the microfluidic reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man, that the use of such microreactors to perform reactions was previously disclosed by Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, and Seki. Further, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001. Exs. 49-51 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 49); Ex. 52 (contemporaneous article by Higuchi entitled "Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media").

A person of ordinary skill in the art would have been further motivated to use two or more aqueous fluids, as described in Kenis, Weigl, Kopf-Sill, Floyd, Johnson, Erbacher, or Whitesides to conduct reactions because the art had already described these concepts. For

example, Quake explicitly contemplates conducting reactions within droplets using two different aqueous fluids. For example, Quake discloses that:

In another preferred embodiment, the device comprises at least two inlet regions, each connecting to the main channel at a droplet extrusion region. In particular, the device may comprise a first inlet region in communication with the main channel at a second droplet extrusion region. A fluid containing a first biological material may pass through the first inlet region so that droplets of the fluid containing the first biological material are sheared into the main channel. A fluid containing a second biological material may pass through the second inlet region so that droplets of the fluid containing the second biological material are sheared into the main channel. In various aspects, the droplets of the first material may mix or combine with the droplets of the second biological material and the first and second biological materials may interact with each other upon mixing. For example, the first biological material may be an enzyme and the second biological material may be a substrate for the enzyme. The interaction of the first and second biological materials may produce a signal that can be detected, *e.g.*, as the droplet passes through a detection region associated with the device.

Ex. 1 at 7:20-8:5; Ex. 2 at ¶ [0018]. Therefore, it was known within the art that using two aqueous fluids to conduct a reaction was useful for separating reagents before droplet formation. *Id.* For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using two or more aqueous fluids to conduct reactions in microfluidic droplets.

A person of ordinary skill in the art would have been further motivated to use oils and surfactants, including fluorinated oils and surfactants, of Ramsey, Parris, Shenderov, Green,

Schubert, Krafft, Delpuech, Paolini, Sadtler, Mason, or Smythe in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ex. 14 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

10X further refers Plaintiffs to the pleadings and briefing in the related *inter partes* review proceedings.

(ii) The '193 Patent

The following combinations render the '193 patent obvious. These combinations are merely exemplary and are not intended to be exhaustive:

- Certain asserted claims are rendered obvious by Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination.
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).

combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).

A person of ordinary skill in the art would have been motivated to combine the references listed in Appendix F, including because these references share common subject matter and approaches, discuss related products and technologies, and/or were developed during the same time period. Most of the references concern conducting reactions by using immiscible carrier fluid to partition aqueous fluid within microfluidic devices. For example, Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials.” Ex. 1 at Abstract; Ex. 2 at Abstract. Droplets are formed using sample fluid that is incompatible with extrusion fluid, within which reactions are conducted. Ex. 1 at 8:14-9:2; Ex. 2 at ¶ [0020]. Quake also explicitly contemplates conducting autocatalytic reactions within microfluidic droplets. For example, Quake states “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1 at 24:12-13; Ex. 2 at ¶ [0080]. Quake also describes conducting a specific reaction using reagents contained in a single aqueous fluid. For example, Quake describes that:

In another embodiment, cells may produce a reporter *in vivo* (*e.g.* a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (*e.g.* benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can

be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate. Sorting can also be done based on a threshold or window concentration of reaction product, which in turn can be correlated with the level of fluorescence. A second reagent or coupling enzyme can be used to enhance fluorescence. *See*, Affholter and Arnold (34) and Joo et al. (35). Any mechanism of this kind, including any reporter or combinations of substrate, enzyme and product can be used for detection and sorting in a like manner, so long as there is at least one way to detect or measure the presence or degree of the reaction of interest.

Ex. 1 at 51:19-52:5; Ex. 2 at [0170]. Corbett pertains to “amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction” in droplets using a “tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample” and reagents. Ex. 6 at 4:24-52.

A person of ordinary skill in the art would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions as taught by Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man. A skilled artisan would have been strongly motivated to perform such reactions in such microfluidic reactors because doing so have provided the substantial benefits known to be associated with microfluidic reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small

volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Ex. 18 at 565. As another example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Ex. 7 at 24. It was also well known that miniaturization of reactions to the microfluidic level provided the substantial advantage of making reactors highly portable, energy efficient, and automated. Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 48 at 1047. Moreover, it was generally known that portable reactors were useful to aid physicians in development of treatment of various conditions. *Id.* For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” *Id.* It was thus known that using the droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to perform reactions advantageously would have allowed reactions to be performed in point of care diagnostic applications. Skilled artisans would also have recognized that performing reaction in the microfluidic reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki may enhance the precision of the reaction relative to then-traditional approaches.

Moreover, a skilled artisan would have fully expected the combination to be successful. The prior art shows that many reactions had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. A skilled artisan would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man.

As further evidence that a skilled artisan would have expected to be successful in the microfluidic reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man, that the use of such microreactors to perform reactions was previously disclosed by Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, and Seki. Further, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001. Exs. 49-51 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 49); Ex. 52 (contemporaneous article by Higuchi entitled "Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media").

A person of ordinary skill in the art would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Parris, Shenderov, Green, Schubert, Delpuech, Paolini, Sadtler, Mason, or Smythe in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and

Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ex. 14 (“Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required.”). For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

10X further refers Plaintiffs to the pleadings and briefing in the related *inter partes* review proceedings.

(iii) The '407 Patent

The following combinations render the '407 patent obvious. These combinations are merely exemplary and are not intended to be exhaustive:

- Certain asserted claims are rendered obvious by Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination.
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination).

A person of ordinary skill in the art would have been motivated to combine the references listed in Appendix G, including because these references share common subject matter and approaches, discuss related products and technologies, and/or were developed during the same time period. Most of the references concern conducting reactions by using immiscible carrier fluid to partition aqueous fluid within microfluidic devices. For example, Quake discloses “a

microfluidic device for analyzing and/or sorting biological materials.” Ex. 1 at Abstract; Ex. 2 at Abstract. Droplets are formed using sample fluid containing “the biological material,” which is incompatible with extrusion fluid and within which reactions are conducted. Ex. 1 at 8:14-9:2; Ex. 2 at ¶ [0020]. Quake also explicitly describes conducting reactions with biological molecules within microfluidic droplets using reagents contained in a single aqueous fluid. For example, Quake states:

In another embodiment, cells may produce a reporter *in vivo* (*e.g.* a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (*e.g.* benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (*e.g.* fluorescent) product is produced from the substrate. Sorting can also be done based on a threshold or window concentration of reaction product, which in turn can be correlated with the level of fluorescence. A second reagent or coupling enzyme can be used to enhance fluorescence. *See*, Affholter and Arnold (34) and Joo et al. (35). Any mechanism of this kind, including any reporter or combinations of substrate, enzyme and product can be used for detection and sorting in a like manner, so long as there is

at least one way to detect or measure the presence or degree of the reaction of interest.

Ex. 1 at 51:19-52:5; Ex. 2 at [0170]. Brown pertains to microfluidic devices for “performing nucleic acid amplification.” Ex. 3 at ¶ [0013]. Corbett pertains to “amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction” in droplets using a “tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample” and reagents. Ex. 6 at 4:24-52.

A person of ordinary skill in the art would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions as taught by Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man. A skilled artisan would have been strongly motivated to perform such reactions in such microfluidic reactors because doing so have provided the substantial benefits known to be associated with microfluidic reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Ex. 18 at 565. As another example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and

preparation of droplets in a microchannel is likely to become increasingly important.” Ex. 7 at 24. It was also well known that miniaturization of reactions to the microfluidic level provided the substantial advantage of making reactors highly portable, energy efficient, and automated. Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 48 at 1047. Moreover, it was generally known that portable reactors were useful to aid physicians in development of treatment of various conditions. *Id.* For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” *Id.* It was thus known that using the droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to perform reactions advantageously would have allowed reactions to be performed in point of care diagnostic applications. Skilled artisans would also have recognized that performing reaction in the microfluidic reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki may enhance the precision of the reaction relative to then-traditional approaches.

Moreover, a skilled artisan would have fully expected the combination to be successful. The prior art shows that many reactions had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. A skilled artisan would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man.

As further evidence that a skilled artisan would have expected to be successful in the microfluidic reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man, that the use of such microreactors to perform reactions was previously disclosed by Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, and Seki. Further, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001. Exs. 49-51 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 49); Ex. 52 (contemporaneous article by Higuchi entitled "Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media").

A person of ordinary skill in the art would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Parris, Shenderov, Green, Schubert, Krafft, Delpuech, Paolini, Sadtler, Mason, or Smythe in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ex. 14 ("Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required."). For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

10X further refers Plaintiffs to the pleadings and briefing in the related *inter partes* review proceedings.

(iv) The '148 Patent

The following combinations render the '148 patent obvious. These combinations are merely exemplary and are not intended to be exhaustive:

- Certain asserted claims are rendered obvious by Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination.
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with

(Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination).

A person of ordinary skill in the art would have been motivated to combine the references listed in Appendix H, including because these references share common subject matter and approaches, discuss related products and technologies, and/or were developed during the same time period. Most of the references concern conducting reactions by using immiscible carrier fluid to partition aqueous fluid within microfluidic devices. For example, Quake discloses “a microfluidic device for analyzing and/or sorting biological materials.” Ex. 1 at Abstract; Ex. 2 at Abstract. Droplets are formed using sample fluid containing “the biological material,” which is incompatible with extrusion fluid and within which reactions are conducted. Ex. 1 at 8:14-9:2; Ex. 2 at ¶ [0020]. Quake also explicitly contemplates conducting PCR within microfluidic droplets. For example, Quake states “[m]icrofabrication permits other technologies to be

integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1 at 24:12-13; Ex. 2 at ¶ [0080]. Quake also describes conducting a specific reaction using reagents contained in a single aqueous fluid. For example, Quake describes that:

In another embodiment, cells may produce a reporter *in vivo* (e.g. a fluorescent compound) through interaction with a reagent added to the fluid medium. For example, cells containing a gene for an oxygenase enzyme may catalyze a reaction on an aromatic substrate (e.g. benzene or naphthalene) with the net result that the fluorescence, or another detectable property of the substrate, will change. This change can be detected in the detection region, and cells having that change in fluorescence can be collected based on predetermined criteria. For example, cells that produce a desired monooxygenase enzyme (such as a P450 enzyme) can be detected in the presence of a suitable substrate (such as naphthalene), and can be collected according to the invention, based on the ability of the enzyme to catalyze a reaction in which a detectable (e.g. fluorescent) product is produced from the substrate. Sorting can also be done based on a threshold or window concentration of reaction product, which in turn can be correlated with the level of fluorescence. A second reagent or coupling enzyme can be used to enhance fluorescence. *See*, Affholter and Arnold (34) and Joo et al. (35). Any mechanism of this kind, including any reporter or combinations of substrate, enzyme and product can be used for detection and sorting in a like manner, so long as there is at least one way to detect or measure the presence or degree of the reaction of interest.

Ex. 1 at 51:19-52:5; Ex. 2 at [0170]. Brown pertains to microfluidic devices for “performing nucleic acid amplification.” Ex. 3 at ¶ [0013]. Corbett pertains to “amplifying the amount of specific target DNA sequence(s) present in a sample using the polymerase chain reaction” in droplets using a “tube adapted to carry a stream of carrier fluid, the tube being provided with an inlet port to enable injection of a reaction mixture comprising the sample” and reagents. Ex. 6 at 4:24-52.

A person of ordinary skill in the art would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions as taught by Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man. A skilled artisan would have been strongly motivated to perform such reactions in such microfluidic reactors because doing so have provided the substantial benefits known to be associated with microfluidic reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Ex. 18 at 565. As another example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Ex. 7 at 24. It was also well known that miniaturization of reactions to the microfluidic level provided the

substantial advantage of making reactors highly portable, energy efficient, and automated. Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 48 at 1047. Moreover, it was generally known that portable reactors were useful to aid physicians in development of treatment of various conditions. *Id.* For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” *Id.* It was thus known that using the droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to perform reactions advantageously would have allowed reactions to be performed in point of care diagnostic applications. Skilled artisans would also have recognized that performing reaction in the microfluidic reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki may enhance the precision of the reaction relative to then-traditional approaches.

Moreover, a skilled artisan would have fully expected the combination to be successful. The prior art shows that many reactions had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. A skilled artisan would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man.

As further evidence that a skilled artisan would have expected to be successful in the microfluidic reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett,

Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man, that the use of such microreactors to perform reactions was previously disclosed by Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, and Seki. Further, in early 2001 a group from the University of Tokyo developed a droplet reactor at least as early as February 23, 2001. Exs. 49-51 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 49); Ex. 52 (contemporaneous article by Higuchi entitled "Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media").

A person of ordinary skill in the art would have been further motivated to use oils and surfactants, including fluorinated oils and fluorinated surfactants, of Ramsey, Parris, Shenderov, Green, Schubert, Krafft, Delpuech, Paolini, Sadtler, Mason, or Smythe in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ex. 14 ("Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required."). For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils and fluorinated surfactants for microfluidic droplet formation.

10X further refers Plaintiffs to the pleadings and briefing in the related *inter partes* review proceedings.

(v) The '083 Patent

The following combinations render the '083 patent obvious. These combinations are merely exemplary and are not intended to be exhaustive:

- Certain asserted claims are rendered obvious by Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination.
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green or Schubert or Krafft, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).
- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in

combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination).

- Certain asserted claims are rendered obvious by (Quake or Shaw Stewart or Shaw Stewart Thesis or Burns (2001) or Nisisako or Thorsen or Seki, alone or in combination) in combination with (Ramsey or Parris or Shenderov or Green, alone or in combination) in combination with (Delpuech or Paolini or Sadtler or Mason or Smythe or Schubert or Krafft, alone or in combination) in combination with (Corbett or Wang or Curcio or Lagally or Burns (1996) or Chiou or Parris or Haff or Brown or Bohm or Chow or Ghadessy or Katsura or Tawfik or Man, alone or in combination).

A person of ordinary skill in the art would have been motivated to combine the references listed Appendix I, including because these references share common subject matter and approaches, discuss related products and technologies, and/or were developed during the same time period. Most of the references concern using oil, including fluorinated oil, to partition aqueous fluid within microfluidic devices. For example, Quake discloses “a microfluidic device for analyzing and/or sorting biological materials.” Ex. 1 at Abstract; Ex. 2 at Abstract. Droplets are formed using sample fluid that is incompatible with extrusion fluid, within which reactions are conducted. Ex. 1 at 8:14-9:2; Ex. 2 at ¶ [0020]. Quake also discloses microchannels “formed from silicon elastomer,” as well as fluorinated oil and fluorinated surfactants. Ex. 1 at 7:11-8:5; Ex. 2 at ¶¶ [0116]-[0118]. Schubert pertains to “[m]icroemulsions containing fluorinated oils . . .

prepared by mixing water, the perfluorinated oil, and a fluorinated surfactant which may be either ionic or non-ionic.” Ex. 31 at Abstract.

A person of ordinary skill in the art would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to conduct reactions as taught by Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man. A skilled artisan would have been strongly motivated to perform such reactions in such microfluidic reactors because doing so have provided the substantial benefits known to be associated with microfluidic reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.” Ex. 18 at 565. As another example, Nisisako describes that “[t]he past decade has seen increased interest in chemical reactions in microfabricated devices. Analysis of reactions in these devices demands technique for handling small amount of liquids, such as metering, transportation and mixing. Recently, chemical and biochemical experiments have been reported using pico/nanoliter-sized droplets. Treating liquid samples in droplet shape has the advantage that dead volume can be decreased. Therefore, a droplet-based chemical reactor is attractive, and preparation of droplets in a microchannel is likely to become increasingly important.” Ex. 7 at 24. It was also well known that miniaturization of reactions to the microfluidic level provided the substantial advantage of making reactors highly portable, energy efficient, and automated. Kopp explained that portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 48 at 1047. Moreover, it was generally known that portable reactors were useful

to aid physicians in development of treatment of various conditions. *Id.* For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” *Id.* It was thus known that using the droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki to perform reactions advantageously would have allowed reactions to be performed in point of care diagnostic applications. Skilled artisans would also have recognized that performing reaction in the microfluidic reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki may enhance the precision of the reaction relative to then-traditional approaches.

Moreover, a skilled artisan would have fully expected the combination to be successful. The prior art shows that many reactions had been successfully achieved in microfluidic devices in the years leading up to the earliest claimed priority date. A skilled artisan would have expected that the microfluidic droplet reactors of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man.

As further evidence that a skilled artisan would have expected to be successful in the microfluidic reactor of Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, Thorsen, or Seki could be successfully modified to perform the reactions disclosed in Corbett, Wang, Curcio, Lagally, Burns (1996), Chiou, Parris, Haff, Brown, Bohm, Chow, Ghadessy, Katsura, Tawfik, or Man, that the use of such microreactors to perform reactions was previously disclosed by Quake, Shaw Stewart, Shaw Stewart Thesis, Burns (2001), Nisisako, and Seki. Further, in early 2001 a group from the University of Tokyo developed a droplet reactor at least

as early as February 23, 2001. Exs. 49-51 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 49); Ex. 52 (contemporaneous article by Higuchi entitled "Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media").

A person of ordinary skill in the art would have been further motivated to use oils and surfactants, including fluorinated oils and surfactants, of Ramsey, Parris, Shenderov, Green, Schubert, Krafft, Delpuech, Paolini, Sadtler, Mason, or Smythe in these microreactor systems to conduct reactions because the art had already described these concepts. For example, Quake disclosed using fluorinated oils and fluorinated surfactants with microfluidic droplets, and Schubert disclosed using fluorinated oils and fluorinated surfactants with microemulsions. A person of skill in the art would have known that generally, fluorinated compounds were biocompatible. *See* Ex. 14 ("Perfluorocarbons may also be suitable because they are widely used where biocompatibility is required."). For these same reasons, a person of skill in the art would have had a reasonable expectation of success in using fluorinated oils, fluorinated surfactants, and non-fluorinated channels for microfluidic droplet formation.

10X further refers Plaintiffs to the pleadings and briefing in the related *inter partes* review proceedings.

IV. Indefiniteness, Enablement, and Written Description

The asserted claims of the Ismagilov patents are also invalid based on indefiniteness under 35 U.S.C. § 112, ¶ 2, lack of written description under 35 U.S.C. § 112, ¶ 1, and/or lack of enablement under 35 U.S.C. § 112, ¶ 1. 10X's contentions that the following claims are invalid under 35 U.S.C. § 112 are made in the alternative, and do not constitute, and should not be interpreted as, admissions regarding the construction or scope of the asserted claims, or that any

of the asserted claims are not anticipated or rendered obvious by any prior art. 10X reserves the right to supplement, amend, and/or modify these disclosures.

1. Written Description

The first paragraph of 35 U.S.C. § 112 requires a patent specification to contain “a written description of the invention.” To satisfy the written description requirement, the description must “clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed.” *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010) (internal citation omitted). “Whether the written description requirement is satisfied is a fact-based inquiry that will depend on the nature of the claimed invention, and the knowledge of one skilled in the art at the time an invention is made and a patent application is filed.” *Carnegie Mellon Univ. v. Hoffmann-La Roche Inc.*, 541 F.3d 1115, 1122 (Fed. Cir. 2008) (internal citation omitted).

10X lists below the grounds upon which it presently contends that certain of the asserted claims of the Ismagilov patents are invalid under 35 U.S.C. § 112, ¶ 1 for failure to provide an adequate written description.

(a) The '091 Patent

The claims of the '091 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because the specification lacks an adequate written description of the alleged invention. Claims of the '091 patent, for example, require a “reaction.” Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a “DNA amplification reaction” in its 10X GemCode platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 7,129,091 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 10, 57, 64, 107, 136, 152, 161, 213, 220, 234, 244, 293, 300,

333, 345, 357, 403, 410. But the specification of the '091 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs' actual and/or apparent application of the claims. There is, for example, no adequate description of performing a "reaction," including, for example, a DNA amplification reaction. There is also, for example, no adequate description of performing a "reaction" outside of a substrate, including, for example, a DNA amplification reaction outside of a substrate. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544, this application lacks adequate description of performing a "reaction," including, for example, a DNA amplification reaction. This application also lacks adequate description of performing a "reaction," outside of a substrate, including, for example, a DNA amplification reaction outside a substrate.

(b) The '193 Patent

The claims of the '193 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because the specification lacks an adequate written description of the alleged invention. Claims of the '193 patent, for example, require an "autocatalytic reaction." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" in its 10X GemCode platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,304,193 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 11, 60, 65, 67, 84-85, 92. But the specification of the '193 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs' actual and/or apparent application of the claims. There is, for example, no adequate description of performing an "autocatalytic reaction," including, for example, a DNA amplification reaction.

There is also, for example, no adequate description of performing an “autocatalytic reaction” outside of a substrate, including, for example, a DNA amplification reaction outside of a substrate. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544, this application lacks adequate description of performing an “autocatalytic reaction,” including, for example, a DNA amplification reaction. This application also lacks adequate description of performing an “autocatalytic reaction” outside of a substrates, including, for example, a DNA amplification reaction outside of a substrate.

As another example, claims of the ’193 patent require “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provid[es] conditions suitable,” which includes “the control of temperature to cycle the DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction,” by, for example, “plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol.” *Id.* at 65-67. But the specification of the ’193 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs’ actual and/or apparent application of the claims. There is, for example, no adequate description of “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified” outside of a microfluidic system or substrate. The claims of the ’193 patent purport to cover all “conditions suitable” for any kind of “autocatalytic reaction,” even though the specification provides no written description adequate to support the breadth of the claim language.

(c) The '407 Patent

The claims of the '407 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because the specification lacks an adequate written description of the alleged invention. Claims of the '407 patent, for example, require a "reaction." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" in its 10X GemCode platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,329,407 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 11, 36, 61, 63, 79. But the specification of the '407 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs' actual and/or apparent application of the claims. There is, for example, no adequate description of performing a "reaction" with a "biological molecule," including, for example, a DNA amplification. There is also, for example, no adequate description of performing a "reaction" with a "biological molecule outside of a substrate, including, for example, a DNA amplification reaction outside of a substrate. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544, this application lacks no adequate description of performing a "reaction" with a "biological molecule," including, for example, a DNA amplification. This application also lacks no adequate description of performing a "reaction" with a "biological molecule" outside of a substrate, including, for example, a DNA amplification outside of a substrate.

As another example, claims of the '407 patent require "providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X "provid[es] conditions suitable," which includes "the control of temperature to cycle the

DNA amplification reaction, the biocompatible conditions within the droplet that allow for enzymes to function, and the appropriate levels of reagents for the DNA amplification reaction,” by, for example, “plac[ing] [the droplets] in a standard 96-well plate and put[ting them] on a thermal cycler for a thermal cycling protocol.” *Id.* at 61-63. But the specification of the ’407 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs’ actual and/or apparent application of the claims. There is, for example, no adequate description of “providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product” outside of a substrate. The claims of the ’407 patent purport to cover all “conditions suitable” for any kind of “reaction,” even though the specification provides no written description adequate to support the breadth of the claim language.

(d) The ’148 Patent

The claims of the ’148 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because the specification lacks an adequate written description of the alleged invention. Claims of the ’148 patent, for example, require “providing conditions suitable for a polymerase-chain reaction.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provides conditions suitable” for the “PCR reaction” by “the control of temperature to cycle the DNA amplification reaction” in its 10X GemCode platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 32-36. But the specification of the ’148 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs’ actual and/or apparent application of the claims. There is, for example, no adequate description of

“providing conditions suitable for a polymerase-chain reaction.” There is also, for example, no adequate description of “providing conditions suitable for a polymerase-chain reaction” outside of a substrate. The claims of the ’148 patent purport to cover all “conditions suitable” for a “polymerase-chain reaction,” even though the specification provides no written description adequate to support the breadth of the claim language. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544, this application lacks adequate description of “providing conditions suitable for a polymerase-chain reaction.” This application also lacks adequate description of “providing conditions suitable for a polymerase-chain reaction” outside of a substrate.

As another example, claims of the ’148 patent require flowing an aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules “under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provides the DNA and reagents under conditions in which they do not react” because “a DNA amplification reaction takes place once the droplets are transferred to a 96-well plate for thermal cycling, where the amplification of the DNA takes place.” *Id.* at 17-19. “During this thermal cycling protocol, oligos which have been released as the gel bead fall apart prime off of the genome and do a low-level of copying.” *Id.* at 18. But the specification of the ’148 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs’ actual and/or apparent application of the claims. There is, for example, no adequate description of “provid[ing] the DNA and reagents under conditions in which they do not react.” The claims of the ’148 patent purport to cover all “conditions” under which any target DNA or RNA

molecule and any other molecule in the fluid “do not react with each other,” even though the specification provides no written description adequate to support the breadth of the claim language.

(e) The '083 Patent

Claims 10, 11, 20-22 and 26 of the '083 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because the specification lacks an adequate written description of the alleged invention. Claim 10, for example, requires a “reagent for an autocatalytic reaction.” Claim 11, for example, requires “a reagent for an autocatalytic reaction” “wherein the autocatalytic reaction is polymerase-chain reaction.” Claims 20-22 and 26 of the '083 patent, for example, require a “reaction.” Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a “DNA amplification reaction” in its 10X GemCode platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,889,083 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 67, 75, 109, 128. But the specification of the '083 patent does not contain a full, clear, concise, and exact written description of how to perform this limitation in this manner, at least under Plaintiffs' actual and/or apparent application of the claims. There is, for example, no adequate description of performing an “autocatalytic reaction,” including, for example, a DNA amplification reaction. There is also, for example, no adequate description of performing an “autocatalytic reaction” outside of a substrate, including, for example, a DNA amplification reaction outside of a substrate. Further, to the extent that Plaintiffs claim priority to U.S. Provisional Application 60/394,544, this application lacks adequate description of performing an “autocatalytic reaction,” including, for example, a DNA amplification reaction. This application also lacks no adequate description of performing an “autocatalytic reaction” outside of a

substrate, including, for example, a DNA amplification reaction outside of a substrate.. For example, the specification of the '083 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the '083 patent does not teach the reactions performed in the accused products.

2. Enablement

To satisfy the enablement requirement of 35 U.S.C. § 112, ¶ 1, the disclosure “must teach those skilled in the art how to make and use the full scope of the claimed invention without ‘undue experimentation.’” *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997). The Federal Circuit has enumerated several factors to consider in determining whether a disclosure would require “undue experimentation”: “(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.” *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

10X lists below the grounds upon which it presently contends that certain of the asserted claims of the Ismagilov patents are invalid under 35 U.S.C. § 112, ¶ 1 for failure to provide an enabling disclosure.

(a) The '091 Patent

The claims of the '091 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because a person of ordinary skill in the art, at the time of the alleged invention, would not have been able to practice those aspects of the asserted claims without undue experimentation. Claims of the '091 patent, for example, require “conducting a reaction within at least one plug.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X performs a “DNA amplification reaction” within plugs. Infringement of U.S.

Patent No. 7,129,091 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 152, 234, 345. But the specification of the '091 patent does not enable the full scope of the limitation, at least under Plaintiffs' actual and/or apparent application of the claims, without undue experimentation. The '091 patent fails to disclose, teach, or suggest how to conduct a "DNA amplification reaction," and particularly, the "DNA amplification reaction" allegedly performed by 10X, within plugs. For example, the specification of the '091 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the '091 patent does not teach the reactions performed in the accused products.

As another example, claims of the '091 patent, for example, require a "conducting a reaction within at least one plug." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" in its 10X GemCode platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 7,129,091 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 10, 57, 64, 107, 136, 152, 161, 213, 220, 234, 244, 293, 300, 333, 345, 357, 403, 410. But the specification of the '091 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. For example, a surfactant that would enable a person of ordinary skill in the art to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate was not available as of the priority date of the '091 patent. In order to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) "provide stability to the drops, prevent coalescence; and (2) "produce a biologically inert interior surface for the water drops." Ex. 53 at 2. Even as of 2008, persons skilled in the art understood that "[b]iological assays thus demand fluorosurfactants with

non-ionic headgroups; however, there are currently no such surfactants available.” *Id.* Therefore, as its priority date, the ’091 patent would not have enabled a person of ordinary skill in the art to perform a “DNA amplification reaction” within “plugs” outside of a microfluidic substrate. *See generally* B. Hindson Depo. Tr., Gerdt’s Depo. Tr., C. Hindson Depo. Tr., Lowe Depo. Tr., Ness Depo. Tr., Price Depo. Tr., Wyatt Depo. Tr., Agresti Depo. Tr. The ’091 patent fails to disclose, teach, or suggest how to conduct a “reaction within at least one plug” outside of a substrate.

(b) The ’193 Patent

The claims of the ’193 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because a person of ordinary skill in the art, at the time of the alleged invention, would not have been able to practice those aspects of the asserted claims without undue experimentation. Claims of the ’193 patent, for example, require “conducting an autocatalytic reaction in plugs.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X performs a “DNA amplification reaction” within plugs. Infringement of U.S. Patent No. 8,304,193 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1. But the specification of the ’193 patent does not enable the full scope of the limitation, at least under Plaintiffs’ actual and/or apparent application of the claims, without undue experimentation. The ’193 patent fails to disclose, teach, or suggest how to conduct a “DNA amplification reaction,” and particularly, the “DNA amplification reaction” allegedly performed by 10X, within plugs. For example, the specification of the ’193 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the ’091 patent does not teach the reactions performed in the accused products.

As another example, claims of the ’193 patent, for example, require an “conducting an autocatalytic reaction in plugs.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X performs a “DNA amplification reaction” in its 10X GemCode platform after the droplets

“com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,304,193 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 1, 11, 60, 65, 67, 84-85, 92. But the specification of the ’193 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. For example, a surfactant that would enable a person of ordinary skill in the art to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate was not available as of the priority date of the ’193 patent. In order to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, prevent coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Ex. 53 at 2. Even as of 2008, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.* Therefore, as its priority date, the ’193 patent would not have enabled a person of ordinary skill in the art to perform a “DNA amplification reaction” within “plugs” outside of a microfluidic substrate. *See generally* B. Hindson Depo. Tr., Gerdt’s Depo. Tr., C. Hindson Depo. Tr., Lowe Depo. Tr., Ness Depo. Tr., Price Depo. Tr., Wyatt Depo. Tr., Agresti Depo. Tr. The ’091 patent fails to disclose, teach, or suggest how to conduct an “autocatalytic reaction in plugs” outside of a substrate.

As another example, claims of the ’193 patent require “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.” But the specification of the ’193 patent does not enable the full scope of this limitation, at least under Plaintiffs’ actual and/or apparent application of the claims, without undue experimentation. The ’193 patent fails to disclose, teach, or suggest how to provide *all* conditions suitable for an autocatalytic reaction.

(c) The '407 Patent

The claims of the '407 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because a person of ordinary skill in the art, at the time of the alleged invention, would not have been able to practice those aspects of the asserted claims without undue experimentation. Claims of the '407 patent, for example, require "conducting a reaction in plugs." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" within plugs. Infringement of U.S. Patent No. 8,329,407 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1. But the specification of the '407 patent does not enable the full scope of the limitation, at least under Plaintiffs' actual and/or apparent application of the claims, without undue experimentation. The '407 patent fails to disclose, teach, or suggest how to conduct a "DNA amplification reaction," and particularly, the "DNA amplification reaction" allegedly performed by 10X, within plugs. For example, the specification of the '407 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the '091 patent does not teach the reactions performed in the accused products.

As another example, claims of the '407 patent, for example, require a "conducting a reaction." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" in its 10X GemCode platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,329,407 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 1, 11, 36, 61, 63, 79. But the specification of the '407 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. For example, a surfactant that would enable a person of ordinary skill in the art to conduct biological assays within stable microfluidic droplets outside of a microfluidic

substrate was not available as of the priority date of the '407 patent. In order to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) “provide stability to the drops, prevent coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Ex. 53 at 2. Even as of 2008, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.* Therefore, as its priority date, the '407 patent would not have enabled a person of ordinary skill in the art to perform a “DNA amplification reaction” within “plugs” outside of a microfluidic substrate. *See generally* B. Hindson Depo. Tr., Gerdt's Depo. Tr., C. Hindson Depo. Tr., Lowe Depo. Tr., Ness Depo. Tr., Price Depo. Tr., Wyatt Depo. Tr., Agresti Depo. Tr. The '407 patent fails to disclose, teach, or suggest how to conduct a “reaction in plugs” outside of a substrate.

As another example, claims of the '407 patent require “providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.” But the specification of the '407 patent does not enable the full scope of this limitation, at least under Plaintiffs’ actual and/or apparent application of the claims, without undue experimentation. The '407 patent fails to disclose, teach, or suggest how to provide *all* conditions suitable for a reaction between the biological molecule and the reagent.

(d) The '148 Patent

The claims of the '148 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because a person of ordinary skill in the art, at the time of the alleged invention, would not have been able to practice those aspects of the asserted claims without undue experimentation. Claims of the '148 patent, for example, require “providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.” Based on Plaintiffs’ 4(c)

disclosures, Plaintiffs contend that 10X “provides conditions suitable” for the “PCR reaction in the plug” by “control[ing] the temperature to cycle the DNA amplification reaction” and the “target DNA or RNA is amplified” through a “PCR reaction.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 32-33. But the specification of the ’148 patent does not enable the full scope of the limitation, at least under Plaintiffs’ actual and/or apparent application of the claims, without undue experimentation. The ’148 patent fails to disclose, teach, or suggest how to provide conditions suitable for conducting a “DNA amplification reaction” and particularly, the “DNA amplification reaction” allegedly performed by 10X, within plugs. For example, the specification of the ’148 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the ’091 patent does not teach the reactions performed in the accused products.

As another example, claims of the ’148 patent, for example, require “providing conditions suitable for a polymerase-chain reaction.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X “provides conditions suitable” for the “PCR reaction” by “the control of temperature to cycle the DNA amplification reaction” in its 10X GemCode platform after the droplets “com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 32-35. But the specification of the ’148 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. For example, a surfactant that would enable a person of ordinary skill in the art to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate was not available as of the priority date of the ’148 patent. In order to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate, a

surfactant was needed to: (1) “provide stability to the drops, prevent coalescence; and (2) “produce a biologically inert interior surface for the water drops.” Ex. 53 at 2. Even as of 2008, persons skilled in the art understood that “[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.” *Id.* Therefore, as its priority date, the ’148 patent would not have enabled a person of ordinary skill in the art to perform a “DNA amplification reaction” within “plugs” outside of a microfluidic substrate. *See generally* B. Hindson Depo. Tr., Gerdt’s Depo. Tr., C. Hindson Depo. Tr., Lowe Depo. Tr., Ness Depo. Tr., Price Depo. Tr., Wyatt Depo. Tr., Agresti Depo. Tr. The ’148 patent fails to disclose, teach, or suggest how to provide “conditions suitable for a polymerase-chain reaction” outside of a substrate.

As another example, claims of the ’148 patent require “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.” Based on Plaintiffs’ 4(c) disclosures, Plaintiffs contend that 10X provides “conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other” because “after the droplets come off the instrument, the reagents inside the droplets do not react. Rather, a DNA amplification reaction takes place once the droplets are transferred to a 96-well plate for thermal cycling.” Infringement of U.S. Patent No. 8,822,148 by 10X’s GemCode Platform (Plaintiffs’ 4(c) disclosures) at 18. “During this thermal cycling protocol, oligos which have been released as the gel bead fall[s] apart prime off of the genome and do a low-level of copying.” *Id.* But the specification of the ’148 patent does not enable the full scope of the limitation, at least under Plaintiffs’ actual and/or apparent application of the claims, without undue experimentation. For example, the specification of the ’148 patent does not teach the use of a dissolvable gel bead to separate reagents contained within the same droplet. The ’148 patent

fails to disclose, teach, or suggest how to provide conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other through the use of a gel bead.

As another example, claims of the '148 patent require a plurality of plugs "each having a substantially uniform size of about 200 μm or less." But the specification of the '148 patent does not enable the full scope of this limitation, at least under Plaintiffs' actual and/or apparent application of the claims, without undue experimentation. The '148 patent fails to disclose, teach, or suggest how to form a plurality of plugs that are smaller than about 200 μm . For example, the '148 patent fails to disclose, teach, or suggest how to form a plurality of plugs that are 0.000000001 μm .

As another example, claims of the '148 patent require a "providing a microfluidic system comprising one or more channels." But the specification of the '148 patent does not enable the full scope of this limitation, at least under Plaintiffs' actual and/or apparent application of the claims, without undue experimentation. The '148 patent fails to disclose, teach, or suggest how to provide a microfluidic system that satisfies the claim limitations comprising *only* one channel.

(e) The '083 Patent

Claims 10, 11, 20-22 and 26 of the '083 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid under 35 U.S.C. § 112, ¶ 1 because a person of ordinary skill in the art, at the time of the alleged invention, would not have been able to practice those aspects of the asserted claims without undue experimentation. Claims 10, 11, 20-22 and 26 of the '083 patent, for example, require either "at least one reagent for an autocatalytic reaction" or "conducting a reaction within at least one plug." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" within plugs. Infringement of U.S. Patent No. 8,889,083 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 67.

But the specification of the '083 patent does not enable the full scope of the limitation, at least under Plaintiffs' actual and/or apparent application of the claims, without undue experimentation. The '083 patent fails to disclose, teach, or suggest how to conduct a "DNA amplification reaction," and particularly, the "DNA amplification reaction" allegedly performed by 10X, within plugs. For example, the specification of the '083 patent does not teach the use of a dissolvable gel bead to accomplish reagent loading. As another example, the specification of the '091 patent does not teach the reactions performed in the accused products.

As another example, claims 10, 11, 20-22 and 26 of the '083 patent, for example, require "conducting a reaction within at least one plug." Based on Plaintiffs' 4(c) disclosures, Plaintiffs contend that 10X performs a "DNA amplification reaction" in its 10X GemCode platform after the droplets "com[e] off the instrument [and] are placed in a standard 96-well plate and put on a thermal cycler for a thermal cycling protocol." Infringement of U.S. Patent No. 8,889,083 by 10X's GemCode Platform (Plaintiffs' 4(c) disclosures) at 74, 75. But the specification of the '083 patent does not enable the full scope of the limitation, as construed by the Court, without undue experimentation. For example, a surfactant that would enable a person of ordinary skill in the art to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate was not available as of the priority date of the '083 patent. In order to conduct biological assays within stable microfluidic droplets outside of a microfluidic substrate, a surfactant was needed to: (1) "provide stability to the drops, prevent coalescence; and (2) "produce a biologically inert interior surface for the water drops." Ex. 53 at 2. Even as of 2008, persons skilled in the art understood that "[b]iological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available." *Id.* Therefore, as its priority date, the '083 patent would not have enabled a POSITA to perform a "DNA

amplification reaction” within “plugs” outside of a microfluidic substrate. *See generally* B. Hindson Depo. Tr., Gerdt's Depo. Tr., C. Hindson Depo. Tr., Lowe Depo. Tr., Ness Depo. Tr., Price Depo. Tr., Wyatt Depo. Tr., Agresti Depo. Tr. The '083 patent fails to disclose, teach, or suggest how to conduct a “reaction within at least one plug” outside of a substrate.

3. Indefiniteness

The second paragraph of 35 U.S.C. § 112 requires that a patent claim “particularly point[] out and distinctly claim[] the subject matter which the applicant regards as the invention.” A patent is indefinite “if its claims, read in light of the patent’s specification and prosecution history, fail to inform, with reasonable certainty, those skilled in the art about the scope of the invention.” *Nautilus, Inc. v. Biosig Instruments, Inc.*, 134 S. Ct. 2120, 2123 (2014).

(a) The '193 Patent

10X lists below the ground upon which it presently contends that certain of the asserted claims of the '193 patent are invalid for indefiniteness under 35 U.S.C. § 112, ¶ 2.

For example, claim 7 of the '193 patent is indefinite insofar as it lacks an antecedent basis with respect to the claim limitation “carrier fluid.” As another example, the claims of the '193 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid as indefinite because the '193 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to “providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.” There is, for example, no adequate description of what constitutes “conditions suitable” for the autocatalytic reaction, which could include a multitude of factors. Without an adequate description of what constitutes “conditions suitable” for the autocatalytic reaction, an artisan could not know whether he or she was practicing the claims. As another example, the claims of the '193 patent, at least under Plaintiffs’ actual and/or apparent application of the

claims, are invalid as indefinite because the '193 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to the claim limitation “substantially surrounded by the immiscible carrier fluid flowing through the channel.” There is, for example, no adequate description of what constitutes “the channel.” The claim references “at least two channels,” a “first channel of the at least two channels,” and a “second channel of the at least two channels,” but does not point out and distinctly claim “the channel” in the claim limitation “substantially surrounded by the immiscible carrier fluid flowing through the channel.”

(b) The '407 Patent

10X lists below the ground upon which it presently contends that certain of the asserted claims of the '407 patent are invalid for indefiniteness under 35 U.S.C. § 112, ¶ 2.

For example, the claims of the '407 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid as indefinite because the '407 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to “providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.” There is, for example, no adequate description of what constitutes “conditions suitable” for the reaction between the biological molecule and the reagent, which could include a multitude of factors. Without an adequate description of what constitutes “conditions suitable” for the reaction between the biological molecule and the reagent, an artisan could not know whether he or she was practicing the claims. As another example, the claims of the '407 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid as indefinite because the '407 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to the claim limitation “substantially surrounded by the immiscible

carrier fluid flowing through the channel.” There is, for example, no adequate description of what constitutes “the channel.” The claim references “at least two channels,” a “first channel of the at least two channels,” and a “second channel of the at least two channels,” but does not point out and distinctly claim “the channel” in the claim limitation “substantially surrounded by the immiscible carrier fluid flowing through the channel.”

(c) The ’148 Patent

10X lists below the ground upon which it presently contends that certain of the asserted claims of the ’148 patent are invalid for indefiniteness under 35 U.S.C. § 112, ¶ 2.

For example, the claims of the ’148 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid as indefinite because the ’148 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to “providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.” There is, for example, no adequate description of what constitutes “conditions suitable” for the polymerase-chain reaction, which could include a multitude of factors. Without an adequate description of what constitutes “conditions suitable” for the polymerase-chain reaction, an artisan could not know whether he or she was practicing the claims.

As another example, the claims of the ’148 patent, at least under Plaintiffs’ actual and/or apparent application of the claims, are invalid as indefinite because the ’148 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to “under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other.” There is, for example, no adequate description of what constitutes the “conditions,” which could include a multitude of factors. Without an adequate

description of what constitutes these “conditions,” an artisan could not know whether he or she was practicing the claims.

(d) The '083 Patent

10X lists below the ground upon which it presently contends that certain of the asserted claims of the '083 patent are invalid for indefiniteness under 35 U.S.C. § 112, ¶ 2.

For example, claims 2 and 26 of the '083 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid as indefinite because the '083 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to “at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.” There is, for example, no adequate description of the meaning of “at least one of a cell, a virion, an enzyme, DNA, and RNA.” For example, the claim limitation fails to point out and distinctly claim whether the claim recites “at least one of” each of the listed categories, including a cell, a virion, an enzyme, DNA, and RNA, or whether the claim requires “at least one of” a cell, a virion, an enzyme, DNA *or* RNA. As another example, the claims of the '083 patent, at least under Plaintiffs' actual and/or apparent application of the claims, are invalid as indefinite because the '083 patent fails to point out and distinctly claim the subject matter that the inventor regards as the invention with respect to the limitation “surface tension at the plug-fluid/microchannel wall interface.” For example, the claims refer to “a non-fluorinated microchannel,” “a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel” and “at least one plug comprising an aqueous plug-fluid in the microchannel.” However, the '083 patent claims fail to point out and distinctly claim what constitutes the “plug-fluid/microchannel wall interface.”

Of Counsel:

David I. Gindler (dgindler@irell.com)
Andrei Iancu (aiancu@irell.com)
Lauren N. Drake (ldrake@irell.com)
Elizabeth C. Tuan (etuan@irell.com)
IRELL & MANELLA LLP
1800 Avenue of the Stars, Suite 900
Los Angeles, CA 90067-4276

Michael H. Strub
Dennis J. Courtney
IRELL & MANELLA LLP
840 Newport Center Drive, Suite 400
Newport Beach, CA 92660
(949) 760-0991

Dated: July 21, 2017

/s/ Jason J. Rawnsley

Frederick L. Cottrell, III (#2555)
Jason J. Rawnsley (#5379)
RICHARDS, LAYTON & FINGER, P.A.
920 North King Street
Wilmington, DE 19801
(302) 651-7700
cottrell@rlf.com
rawnsley@rlf.com

Attorneys for 10X Genomics, Inc.

CERTIFICATE OF SERVICE

I hereby certify that on July 21, 2017, I caused true and correct copies of the foregoing document to be served on the following counsel in the manner indicated:

VIA EMAIL

Brian E. Farnan
Michael Farnan
FARNAN LLP
919 N. Market St., 12th Floor
Wilmington, DE 19801
(302) 777-0300
bfarnan@farnanlaw.com
mfarnan@farnanlaw.com

VIA EMAIL

Edward R. Reines
Derek C. Walter
WEIL, GOTSHAL & MANGES LLP
201 Redwood Shores Parkway
Redwood Shores, CA 94065
(650) 802-3000
raindance10xservice@weil.com

/s/ Jason J. Rawnsley

Jason J. Rawnsley (#5379)
rawnsley@rlf.com

Appendix A – Ismagilov References

Description / Title		Author / Inventor
U.S. PATENTS		
1.	2,797,149	Skeggs et al.
2.	3,047,367	Kessler
3.	3,479,141 (“Smythe US 3479,141”)	Smythe et al.
4.	3,960,187	Stock et al.
5.	4,253,846 (“Smythe US 4,253,846”)	Smythe et al.
6.	4,341,867	Johansen
7.	4,853,336	Saros et al.
8.	4,963,498	Hillman et al.
9.	5,185,099 (“Delpuech”)	Delpuech et al.
10.	5,270,183 (“Corbett”)	Corbett et al.
11.	5,585,069	Zanzucchi et al.
12.	5,726,404	Brody
13.	5,739,036 (“Parris”)	Parris
14.	5,837,200	Diessel et al.
15.	5,842,787 (“Kopf-Sill”)	Kopf-Sill et al.
16.	5,846,727	Soper et al.
17.	5,872,010	Karger et al.
18.	5,887,755	Hood, III
19.	5,980,936 (“Krafft”)	Krafft et al.
20.	5,997,636	Gamarnik et al.
21.	6,033,880 (“Haff”)	Haff et al.
22.	6,057,149	Burns et al.
23.	6,118,849	Tanimori et al.
24.	6,130,098	Handique et al.
25.	6,140,053	Koster
26.	6,171,850	Nagle et al.
27.	6,180,372	Franzen
28.	6,296,020	McNeely et al.

Description / Title		Author / Inventor
29.	6,296,673	Santarsiero et al.
30.	6,303,343	Kopf-Sill
31.	6,306,590	Mehta et al.
32.	6,379,929	Burns et al.
33.	6,409,832	Weigl et al.
34.	6,432,290	Harrison et al.
35.	6,444,461	Knapp et al.
36.	6,524,456 (“Ramsey”)	Ramsey et al.
37.	6,569,631	Pantoliano et al.
38.	6,630,006	Santarsiero et al.
39.	6,656,267	Newman, Janet
40.	6,766,817	Silva
41.	6,790,328	Jacobson et al.
42.	6,797,056	David
43.	6,872,250	David et al.
44.	6,880,576	Karp et al.
45.	7,041,481	Anderson et al.
46.	7,129,091	Ismagilov et al.
47.	7,156,917	Moriyama et al.
48.	7,294,503	Quake et al.
U.S. PATENT APPLICATIONS		
49.	U.S. Provisional Appl. No. 60/233,037 (“Quake”)	Quake et al.
50.	U.S. Provisional Appl. No. 60/379,927	da Silva
51.	U.S. Provisional Appl. No. 60/394,544	Ismagilov
U.S. PATENT APPLICATION PUBLICATIONS		
52.	2002/0160518	Hayenga
53.	2001/0036669	Jedrzejewski
54.	2001/0041357	Fouillet et al.
55.	2001/0048900	Bardell et al
56.	2002/0001856	Chow

Description / Title		Author / Inventor
57.	2002/0005354	Spence et al.
58.	2002/0008029	Williams et al.
59.	2002/0012971	Mehta
60.	2002/0017464	Parce et al.
61.	2002/0022261	Anderson et al.
62.	2002/0043463 (“Shenderov”)	Shenderov
63.	2002/0051971	Stuelpnagel et al.
64.	2002/0058332 (“Quake”)	Quake et al.
65.	2002/0067800	Newman et al.
66.	2002/0113009	O’Connor et al.
67.	2002/0119536	Stern
68.	2002/0131147 (“Paolini”)	Paolini
69.	2002/0160518	Hayenga et al.
70.	2002/0164820 (“Brown”)	Brown
71.	2002/0166582	O’Connor et al.
72.	2002/0175079	Christel et al.
73.	2002/0195463 (“Seki”)	Seki et al.
74.	2002/0197630	Knapp et al.
75.	2003/0005967	Karp
76.	2003/0007898 (“Bohm”)	Bohm et al.
77.	2003/0036206	Chien et al.
78.	2003/0061687	Hansen et al.
79.	2003/0064414	Benecky et al.
80.	2003/0075446	Culbertson et al.
81.	2003/0089605	Timperman
82.	2003/0124509	Kenis et al.
83.	2003/0229376	Sandhu
84.	2003/0230486	Chen et al.
85.	2004/0005582	Shipwash
86.	2004/0024380	Chou et al.

Description / Title		Author / Inventor
87.	2004/0037813	Simpson et al.
88.	2004/0068019 (“Higuchi I”)	Higuchi et al.
89.	2004/0136497	Meldrum et al.
90.	2004/0181131	Maynard et al.
91.	2004/0188254	Spaid
92.	2004/0224419	Zheng et al.
93.	2004/0258203	Yamane et al.
94.	2005/0170431	Ibrahim et al.
95.	2006/0094119	Ismagilov et al.
96.	2007/0026439	Faulstick et al.
97.	2008/0014589	Link
98.	2009/0021728	Heinz et al.
99.	2009/0060797	Mathies et al.
100.	2010/0022414	Link et al.
101.	2011/0000560	Miller et al.
INTERNATIONAL PATENT APPLICATIONS		
102.	WO00/21666 PCT	Chow et al.
103.	WO 00/23181 (“Wang”)	Wang et al.
104.	WO 00/43766	Alajoki et al.
105.	WO 01/02850 (“Chow”)	Chow et al.
106.	WO 01/12327 A1 02/22/2001 WI PO	Ramsey, J. M. et al.
107.	WO 01/27610	Chapman et al.
108.	WO 01/64332 A1 09/07/2001 WI PO	Burns
109.	WO 01/77683 A1 10/18/01 PCT	Chow et al.
110.	WO 02/12856 A1 02/14/02 PCT	Parce et al.
111.	WO 02/18949	Fulwyler
112.	WO 02/23163 A1 03/21/2002 PCT	Quake et al.
113.	WO 02/25243 A 1 03/28/02 PCT	Van Gelder
114.	WO 03/044221 A 1 05/30/03 PCT	Timperman
115.	WO 07/021343 A2 02/22/2007 PCT	Baxter et al.

Description / Title		Author / Inventor
116.	WO 84/02000 ("Shaw Stewart")	Shaw Stewart
117.	WO 94/18218	Ulmer
118.	WO 94/19101	Kaler et al.
119.	WO 97/29508 08/14/97 PCT	Carson et al.
120.	WO 98/02237	Lemaitre et al.
121.	WO 98/22625	Burns et al.
122.	WO 98/52691	Harrison et al.
FOREIGN PATENTS		
123.	GB 2,097,692 ("Shaw Stewart")	Shaw Stewart
124.	CN2482070	
125.	EP 0 816 837 01/07/1998 EPO	Chow et al.
126.	EP 0 912 238 81 10/10/2001 Europe	Lemaitre et al.
127.	JP2001145486 (A)	Kojima
128.	JP2001502790A	Bousse et al.
129.	JP2001181309 (A)	Nakajima et al.
130.	JP2003071261 (A)	Nakajima et al.
131.	JP2001048097 (A) ("Higuchi II")	Higuchi et al.
132.	JP2001238624 (A) ("Higuchi III")	Higuchi et al.
NON-PATENT LITERATURE		
133.	A Closed-Cycle Capillary Polymerase Chain Reaction Machine, <i>Anal. Chem.</i> , 73: 2018-2021 (2001) ("Chiou")	Chiou et al.
134.	A Microreactor for the Nitration of Benzene and Toluene (2002)	Burns et al.
135.	A Numerical Model for Segmented Flow in a Microreactor, <i>International Journal of Heat and Mass Transfer</i> , 46: 3313-3322 (2003)	Harries, N. et al.
136.	A Robust and Scalable Microfluidic Metering Method That Allows Protein Crystal Growth by Free Interface Diffusion, <i>PNAS</i> , 99(26): 16531-16536 (2002)	Hansen et al.

Description / Title		Author / Inventor
137.	A sensitive competitive ELISA for 2,4-dinitrophenol using 3,6-fluorescein diphosphate as a fluorogenic substrate, <i>Journal of Immunological Methods</i> , 149: 261-266 (1992)	Huang et al.
138.	A Stable Nonfluorescent Derivative of Resorufin for the Fluorometric Determination of Trace Hydrogen Peroxide: Applications in Detecting the Activity of Phagocyte NADPH Oxidase and Other Oxidases, <i>Analytical Biochemistry</i> , 253: 162-168 (1997)	Zhou et al.
139.	A super-saturation wave of protein crystallization, <i>J. Crystal Growth</i> , 232: 149-155 (2001)	Garcia-Ruiz et al.
140.	Abaxis Company History, as accessed on March 18, 2016, from http://www.abaxis.com/about_us/history.html	
141.	Accuracy of the i-STAT TM bedside blood gas analyser, <i>Eur. Respir. J.</i> , 14, 214-217 (1999)	Sedjame et al.
142.	An Apparatus for Sequentially Combining Microvolumes of Reagents by Infrasonic Mixing, <i>Analytica Biochemistry</i> , 138, 329-334 (1984)	Camien et al.
143.	An Integrated Nanoliter DNA Analysis Device, <i>Science</i> 282: 484-487 (1998)	Burns et al.
144.	An interlaboratory comparison of measurements from filament-stretching rheometers using common test fluids, <i>J. Rheol.</i> , 45(1): 83-114 (2001)	Anna et al.
145.	Application of a Multichannel Dropping Dispenser in Segmented Continuous Flow Analysis, <i>Talanta</i> , 39(2): 95-99 (1992)	Kuban
146.	Artificial Blood, <i>Science</i> , 295: 1002-1005 (2002)	Squires
147.	Biochemistry, Saunders College Publishing 2d ed. (1999)	Garrett et al.
148.	Biocompatible surfactants for water-in-fluorocarbon emulsions, <i>Lab Chip</i> , 8: 1632-1639 (2008)	Holtze et al.
149.	Biotechnology at Low Reynolds Numbers, <i>Biophysical Journal</i> , 71 (1996)	Brody et al.

Description / Title		Author / Inventor
150.	Blood substitutes: Artificial oxygen carriers: perfluorocarbon emulsions, <i>Critical Care</i> , 3: R93-R97 (1999)	Spahn
151.	Capillary-based fully integrated and automated system for nanoliter polymerase chain reaction analysis directly from cheek cells, <i>J. Chromatogr. A</i> , 924, 271-284 (2001)	He et al.
152.	Capillary experiments of flow induced crystallization of HDPE, <i>AIChE Journal</i> , 36(1): 13-18 (1990)	Titomanlio et al.
153.	Cellectricon Company History, as accessed on April 5, 2016, from http://www.cellectricon.com/about-us/our-journey/	
154.	Chaotic Mixer for Microchannels, <i>Science</i> , 295: 647-651 (2002)	Stroock et al.
155.	Chapter 19: Disperse Systems, in Remington's Pharmaceutical Sciences 18 th Ed., Gennaro, A.R. (ed), Mack Publishing Company (1990)	Zografi et al.
156.	Chapter 8: Enzymes in Water-in-oil Microemulsions ('Reversed Micelles'): Principles and Applications, 255-327	Oldfield
157.	Chapter 8: Fluorinated surfactants in practice, in "Design and Selection of Performance Surfactants," Karsa D.R. (ed.), Sheffield Academic Press (1999)	Taylor
158.	Chemical Amplification: Continuous-Flow PCR on a Chip, <i>Science</i> , 280: 1046-1048 (1998) ("Kopp")	Kopp et al.
159.	Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media, the Royal Society of Chemistry (2002)	Taniguchi et al.
160.	Coalescence of Liquid Drops, <i>J. Fluid Mech.</i> , 401: 293-310 (1999)	Eggers et al.
161.	Commercialization of microfluidic point-of-care diagnostic devices, <i>Lab on a Chip</i> , 12: 2118-2134 (2012)	Chin et al.

Description / Title		Author / Inventor
162.	Comparative 90-Day Feeding Study with Low-Viscosity White Mineral Oil in Fischer-344 and Sprague-Dawley-Derived CRL:CD Rats, <i>Toxicologic Pathology</i> , 23(1): 26-33 (1995)	Firriolo et al.
163.	Components for integrated poly(dimethylsiloxane) microfluidic systems, <i>Electrophoresis</i> , 23: 3461-3473 (2002)	Ng et al.
164.	Contamination-Free Continuous Flow Microfluidic Polymerase Chain Reaction for Quantitative and Clinical Applications, <i>Anal. Chem.</i> , 77: 3700-3704 (2005)	Dorfman et al.
165.	Continuous-Flow Chemical Processing on a Microchip by Combining Microunit Operations and a Multiphase Flow Network, <i>Analytical Chemistry</i> , 74(7): 1565-1571 (2002)	Tokeshi et al.
166.	Control and Applications of Immiscible Liquids in Microchannels, <i>J. Am. Chem. Soc.</i> , 124: 5284-5285 (2002)	Zhao et al.
167.	Controlled Production of Emulsions Using a Crossflow Membrane, <i>Particle & Particle Systems Characterization</i> , 15: 21-25 (1998)	Peng et al.
168.	Cost-effective screening methods for various single gene defects in single cells using high magnesium and total ionic strength and restriction enzymes,” <i>Prenat. Diagn.</i> 20: 979-985 (2000)	Tsai
169.	CRC Handbook of Chemistry and Physics, 72 nd Edition 1991-1992	Lide
170.	CRC Handbook of Chemistry and Physics, 80 th Edition 1999-2000	Lide
171.	Crystallization with oils: a new dimension in macromolecular crystal growth, <i>Journal of Crystal Growth</i> , 196: 434-441 (1999)	Chayen
172.	Detection of single molecules: solution-phase single-molecule fluorescent correlation spectroscopy as an ultrasensitive, rapid and reliable system for immunological investigation, <i>Journal of Immunological Methods</i> 260 (2002)	Foldes-Papp

Description / Title		Author / Inventor
173.	Determination of Subyoctomole Amounts of Nonfluorescent Molecules Using a Thermal Lens Microscope: subsingle-Molecule Determination, <i>Anal. Chem.</i> , 73: 2112-2116 (2001)	Tokeshi et al.
174.	Dictionary of Scientific and Technical Terms, <i>McGraw-Hill</i> 5 th Ed., 1498 (1994)	Parker
175.	Digital PCR, <i>Proc. Natl. Acad. Sci. USA, Genetics</i> , 96: 9236-9241 (1999) (“Vogelstein”)	Vogelstein et al.
176.	Directed evolution of polymerase function by compartmentalized self-replication, <i>PNAS</i> , 98: 4552-4557 (2001) (“Ghadessy”)	Ghadessy et al.
177.	DNA amplification with a microfabricated reaction chamber, Transducers '93, Seventh International Conference on Solid State Sensors and Actuators, IEEE Proceedings Yokohama, Japan, 924-926 (1993)	Northrup et al.
178.	Droplets Formation and Merging in Two-Phase Flow Microfluidics, <i>Int. J. Mol. Sci.</i> , 12: 2572-2597 (2011)	Gu et al.
179.	Droplet formation in a microchannel network, <i>Lab on a Chip</i> , 2: 24-26 (2002) (“Nisisako”)	Nisisako et al.
180.	Droplet Formation in a Microchannel on PMMA Plate (“Nisisako Abstract”)	Nisisako et al.
181.	Droplet Manipulation on a Superhydrophobic Surface for Microchemical Analysis, Transducers '01 Eurosensors XV, 11 th International Conference on Solid-State Sensors and Actuators, Munich, Germany, Digest of Technical Papers Vol. 2: 1150-1153 (2001)	Torkkeli et al.
182.	Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device, <i>Phys. Rev. Lett.</i> , 86:4163-4166 (2001) (“Thorsen”)	Thorsen et al.
183.	Dynamic percolation in fluorinated water-in-oil microemulsions, <i>Physical Review</i> , 56(4): 4356-4363 (1977)	Gamber et al.
184.	Effect of Channel Structure on Microchannel Emulsification, <i>Langmuir</i> , 18: 5708-5712 (2002)	Sugiura et al.

Description / Title		Author / Inventor
185.	Effect of Emulsifier Concentration and Type on the Particle Size Distribution of Emulsions, <i>J. Pharm. Sci.</i> , 260-264 (1965)	Rowe
186.	Effect of Microchannel Structure on Droplet Size During Crossflow Microchannel Emulsification, <i>Journal of Surfactants and Detergents</i> , 3(3): 295-302 (2000)	Kawakatsu et al.
187.	Effects of Viscosity on Droplets and Mixing in Multiphase Microfluidics at Low Values of the Reynolds and the Capillary Numbers, <i>Langmuir</i> , 19: 927-9133 (2003)	Tice
188.	Effects of Viscosity on Droplet Formation and Mixing in Microfluidic Channels, <i>Analytica Chimica Acta</i> , vol. 507: 73-77 (2004)	Tice
189.	Efficient Mixing and Reactions Within Microfluidic Channels Using Microbead-Supported Catalysts", JACS Communications, pp. 13360-13361, Published on the Web 10/17/2002.	Seong et al.
190.	Elasto-capillary thinning and breakup of model elastic liquids, <i>J. Rheol.</i> , 45(1): 115- 138 (2001)	Anna et al.
191.	Electrical power free, low dead volume, pressure-driven pumping for microfluidic applications	Moscovici et al.
192.	Electrokinetic displacement of air bubbles in microchannels, <i>Physics of Fluids</i> , 14(1): 1-14 (2002)	Takhistov et al.
193.	Electrowetting-based actuation of droplets for integrated microfluidics, <i>Lab Chip</i> , 2: 96-101 (2002)	Pollack et al.
194.	Enhancement of Mixing By Droplet-Based Microfluidics, 2002 Institute of electrical Engineers 151 h International Conference on Micro Electro Mechanical Systems, pp. 97-100(2002)	Fowler et al.
195.	Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia, <i>Science</i> , 230: 1350-1354 (1985)	Saiki et al.

Description / Title		Author / Inventor
196.	Ethanol Production from Starch by a Coimmobilized Mixed Culture System of <i>Aspergillus awamori</i> and <i>Zymomonas mobilis</i> , <i>Biotechnology and Bioengineering</i> , XXVII: 1761-1768 (1986)	Tanaka et al.
197.	Experimental and theoretical scaling laws for transverse diffusive broadening in two-phase laminar flows in microchannels, <i>Applied Physics Letters</i> , 76(17): 24 (2000)	Ismagilov et al.
198.	Expression of a human placental alkaline phosphatase gene in transfected cells: Use as a reporter for studies of gene expression, <i>Proc. Natl. Acad. Sci. USA</i> , 85: 6342-6346 (1988)	Henthorn et al.
199.	Fabrication inside Microchannels Using Fluid Flow, <i>Accounts of Chemical Research</i> , a Publication of the American Chemical Society, 33(11) (2000) ("Whitesides")	Kenis et al.
200.	Fabrication of Microchambers Defined by Photopolymerized Hydrogels and Weirs within Microfluidic Systems: Application to DNA Hybridization, <i>Analytical Chemistry</i> , 74(14): 3372-3377 (2002)	Seong et al.
201.	Fabrication of Microfluidic Systems in Poly{dimethylsiloxane}, <i>Electrophoresis</i> , 21: 27-40 (2000)	McDonald et al.
202.	Fine Chemical Manipulations of Microscopic Liquid Samples. 1. Droplet Loading with Chemicals	Gratzl et al.
203.	Flexible Methods for Microfluidics, <i>Physics Today</i> , 42-48 (2001)	Whitesides et al.
204.	Fluid Mechanics Fundamentals and Applications, McGraw-Hill (2006)	Cengel et al.
205.	Fluidigm Company History, as accessed on April 5, 2016, from http://www.fluidigm.com/about/aaboutfluidigm	
206.	Fluorescein Product Information, Sigma-Aldrich	
207.	Fluorinated Alkyl Surfactants, <i>Environmental Engineering Science</i> , 20: 487-501 (2003)	Schultz

Description / Title		Author / Inventor
208.	Formation of Dispersions Using ‘Flow Focusing’ in Microchannels, <i>Applied Physics Letters</i> , 82(3): 364-366 (2003)	Anna et al.
209.	Formation of Droplets and Mixing in Multiphase Microfluidics at Low Values of the Reynolds and the Capillary Numbers, <i>Langmuir</i> , 19: 9127-9133 (2003)	Tice et al.
210.	Formation of Droplets of Alternating Composition in Microfluidic Channels and Applications to Indexing of Concentrations in Droplet-Based Assays, <i>Anal. Chem.</i> , 76: 4977-4982 (2004)	Zheng et al.
211.	Formation of Droplets Using Branch Channels in a Microfluidic Circuit, <i>Sice</i> , 1262-1264 (2002)	Nisisako et al.
212.	Generation of Steady Liquid Microthreads and Micron-Sized Monodisperse Sprays in Gas Streams, <i>Physical review Letters</i> , 80(2): 285-288 (1998)	Gañán-Calvo
213.	Google Scholar search results for “perfluorocarbon fluids”, as accessed on April 7, 2016, from http://www.scholar.google.com	
214.	Gordon Research Conferences – 1997 Meeting Program – Bioanalytical Sensors, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=4366	
215.	Gordon Research Conferences – 1998 Meeting Program – Chemical Sensors & Interfacial Design, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=4523	
216.	Gordon Research Conferences – 1999 Meeting Program – Analytical Chemistry, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=7636	
217.	Gordon Research Conferences – 2000 Meeting – Chemical Sensors & Interfacial Design, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=9200	

Description / Title		Author / Inventor
218.	Gordon Research Conferences – 2001 Meeting – Chemical Sensors & Interfacial Design, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=10286	
219.	Gordon Research Conferences – 2001 Meeting – Microfluidics, Physics & Chemistry of, as accessed on March 30, 2016 from http://www.grc.org/programs.aspx?id=10251	
220.	Handling of Picaliter Liquid Samples in a Poly(dimethylsiloxane)- Based Microfluidic Device, <i>Analytical Chemistry</i> , 71(20): 4781-4785 (1999)	Hosokawa et al.
221.	Hawley’s Condensed Chemical Dictionary, Fourteenth Edition	Lewis
222.	Heterogeneous Microfluidic Contacting for Encapsulation of Quantum Dots, <i>A Novel Microchemical System for Rapid Liquid-Liquid Chemistry</i> , Doctoral Dissertation, Massachusetts Institute of Technology, 86-102 (2002) (“Floyd”)	Floyd
223.	Shaw Stewart, The Development and Applications of a New Liquid Handling Device, Doctoral Thesis, University of London and the Imperial College of Science and Technology (1998) (“Shaw Stewart Thesis”)	Shaw Stewart
224.	High Speed Polymerase Chain Reaction in Constant Flow, Bioscience, <i>Biotechnology and Biochemistry</i> , 58(2): 349-352	Nakano et al.
225.	Hydrodynamic Focusing on a Silicon Chip: Mixing Nanoliters in Microseconds, <i>Physical Review Letters</i> , 80(17): 3863-3866 (1998)	Knight
226.	Hydrodynamic Focusing on a Silicon Chip: Mixing Nanoliters in Microseconds, <i>Physical Review Letters</i> , 9(2): 190-197 (2000)	Knight
227.	Improved Techniques for High-Throughput Molecular Diagnostics, Royal Institute of Technology (2002) (“Curcio”)	Curcio
228.	Indirect micromanipulation of single molecules in water-in-oil emulsion, <i>Electrophoresis</i> , 22: 289-293 (2001) (“Katsura”)	Katsura

Description / Title		Author / Inventor
229.	Interfacial properties and emulsion stability in fluorinated oil-non-fluorinated oil-surfactant(s) systems, <i>Colloids and Surfaces</i> , 67: 81-93 (1992)	Morita et al.
230.	Interfacial Tension Driven Monodispersed Droplet Formation from Microfabricated Channel Array, <i>Langmuir</i> , 17: 5562-5566 (2001)	Sugiura et al.
231.	International Symposium on Microchemistry and Microsystems	Kitamori
232.	Introduction to Biostatistics (Dover Publications, Inc. 2009) (1969)	Sokal et al.
233.	Investigation on protein crystal growth by the gel acupuncture method, <i>Acta. Cryst.</i> , 50(99): 484-490 (1994)	Garcia-Ruiz et al.
234.	Laminar-Flow Fluid Mixer for Fast Fluorescence Kinetics Studies, <i>Biophysical Journal</i> , 83: 2872-2878 (2002)	Pabit et al.
235.	Lithographically induced self-Assembly of microstructures with a liquid-filled gap between the mask and polymer surface, <i>J. Vac. Sci. Technol.</i> , B 19(6) (2001)	Deshpande et al.
236.	Marangoni effects of trace impurities on the motion of long gas bubbles in capillaries, <i>J. Fluid Mech.</i> , 210: 303-328 (1990)	Ratulowski et al.
237.	Mathematical modeling of drop mixing in a slit-type microchannel, <i>J. Micromech. Microeng.</i> , 11: 548-554 (2001)	Handique et al.
238.	Micro Total Analysis Systems. 1. Introduction, Theory and Technology, <i>Anal. Chem.</i> , 74: 2623-2636 (2002)	Reyes et al.
239.	Man-made cell-like compartments for molecular evolution, <i>Nature Biotechnology</i> , 16: 652-656 (1998) ("Tawfik")	Tawfik et al.
240.	Melting Points of Linear-Chain Homologs, The Normal Paraffin Hydrocarbons, 85: 3548-3553 (1963)	Flory et al.
241.	Micro Total Analysis Systems. 1. Introduction, Theory and Technology, <i>Analytical Chemistry</i> , 74(12): 2623-2636 (2002)	Reyes et al.

Description / Title		Author / Inventor
242.	Micro Total Analysis Systems. 2. Analytical Standard Operations and Applications, <i>Analytical Chemistry</i> , 74(12): 2637-2652 (2002)	Auroux et al.
243.	Microemulsifying fluorinated oils with mixtures of fluorinated and hydrogenated surfactants, <i>Colloids and Surfaces A: Physicochemical and Engineering Aspects</i> , 84: 97-106 (1994) (“Schubert”)	Schubert et al.
244.	Microfabricated Devices for Sizing DNA and Sorting Cells, <i>Micro- and Nanofabricated Structures and Devices for Biomedical Environmental Applications</i> 3258: 181-187 (1998)	Chou et al.
245.	Microfabricated structures for integrated DNA analysis, <i>Proc. Natl. Acad. Sci. USA</i> , 93: 5556-5561 (1996) (“Burns (1996)”)	Burns et al.
246.	Microfabrication Inside Capillaries Using Multiphase Laminar Flow Patterning (“Kenis”)	Kenis et al.
247.	Microfluidic devices fabricated in poly(dimethylsiloxane) for biological studies, <i>Electrophoresis</i> , 24: 3563-3576 (2003)	Sia et al.
248.	Microfluidic Diffusion-Based Separation and Detection, <i>Science</i> , 283, 15 (1999) (“Weigl”)	Weigl et al.
249.	Microfluidics – a review, <i>J. Micromech. Microeng.</i> , 3: 168-182 (1993)	Gravesen
250.	Microfluidics and point-of-care testing, <i>Lab Chip</i> . 8: 1982-1983 (2008)	Sia et al.
251.	Microfluidic flow focusing: Drop size and scaling in pressure <i>versus</i> flow-rate-driven pumping, <i>Electrophoresis</i> , 26: 3716-3724 (2005)	Ward et al.
252.	Microfluidics: Fluid physics at the nanoliter scale, <i>Reviews of Modern Physics</i> , 77: 977-1026 (2005)	Squires et al.
253.	Microfluidic Large-Scale Integration, <i>Science</i> , 298: 580-584 (2002)	Thorsen et al.
254.	Microfluidic Routes to the Controlled Production of Nanoparticles, <i>Chemical Communications</i> , 1136-1137 (2002)	Edel et al.

Description / Title		Author / Inventor
255.	Microfluidic System for Chemical Kinetics That Rely on Chaotic Mixing in Droplets, <i>Phil. Trans. R. Soc. Lond.</i> , 1-18 (2004)	Bringer et al.
256.	Millisecond Kinetics on a Microfluidic Chip Using Nanoliters of Reagents, <i>J. Am. Chem. Soc.</i> , 125: 14613-14619 (2003)	Song et al.
257.	Miniaturized total analysis systems for biological analysis, <i>Fresenius J. Anal. Chem.</i> , 366: 525-539 (2000)	Jakeway et al.
258.	Miniaturized Total Chemical Analysis Systems: a Novel Concept for Chemical Sensing, <i>Sensors and Actuators B1</i> , 244-248 (1990)	Manz et al.
259.	Mixing with bubbles: a practical technology for use with portable microfluidic devices, <i>Lab Chip</i> , 6: 207-212 (2006)	Garstecki et al.
260.	Monodisperse Emulsion Generation via Drop break Off in a Coflowing Stream, <i>Langmuir</i> , 16: 347-351 (2000)	Umbanhowar et al.
261.	Monolithic Capillary Electrophoresis Device with Integrated Fluorescence Detector, <i>Anal. Chem.</i> , 73: 1622-1626 (2001)	Webster et al.
262.	Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system, <i>Sensors and Actuators B: Chemical</i> , 63(3): 136-146 (2000)	Lagally et al.
263.	Monolithic Integrated PCR Reactor-CE System for DNA Amplification and Analysis to the Single Molecule Limit, 2 nd Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine & Biology, IEEE (2002)	Lagally et al.
264.	Monolithic Structures for Integrated Microfluidic Analysis, Dissertation (2001) ("Man")	Man
265.	Multi-Step Synthesis of Nanoparticles Performed on Millisecond Time Scale in a Microfluidic Droplet-Based System, <i>The Royal Society of Chemistry</i> , 4: 316-321 (2004)	Shestopalov et al.

Description / Title		Author / Inventor
266.	My Bibliography - My NCBI Collection, as accessed on March 6, 2016 from http://www.ncbi.nlm.nih.gov/sites/myncbi/1V_xg_h5XBgH5X/bibliography/48097030/public/?sort=date&direction=ascending	Sia
267.	Nanoliter Liquid Metering in Microchannels Using Hydrophobic Patterns, <i>Anal. Chem.</i> , 72: 4100-4109 (2000)	Handique et al.
268.	Nanoliter scale PCR with TaqMan detection, <i>Nucleic Acids Research</i> , 25(10): 1999-2004 (1997)	Kalinina et al.
269.	Novel Approach for Assessing Performance of PCR Cyclers Used for Diagnostic Testing,” <i>J. of Clin. Microbiol.</i> , 43(6), 2724-2728 (2005)	Schroder et al.
270.	Oligonucleotides with Fluorescent Dyes at Opposite Ends Provide a Quenched Probe System Useful for Detecting PCR Product and Nucleic Acid Hybridization,” <i>Genome Res.</i> , 4: 357-362 (1995)	Livak et al.
271.	On-Chip, Real-Time, Single-Copy Polymerase Chain Reaction in Picoliter Droplets, <i>Anal. Chem.</i> , 79: 8471-8475 (2007)	Beer et al.
272.	On-Chip Thermopneumatic Pressure for Discrete Drop Pumping, <i>Analytical Chemistry</i> , 73: 1831-1838 (2001)	Handique et al.
273.	Operation of Mixed-Culture Immobilized Cell Reactors for the Metabolism of Meta- and Para-Nitrobenzoate by Comamonas Sp. JS46 and Comamonas Sp. JS47, <i>Biotechnology and Bioengineering</i> , 59(1): 21-27 (1998)	Goodall et al.
274.	Passive Mixing in a a Three-Dimensional Serpentine Microchannel, <i>Journal of Microelectromechanical Systems</i> , 9(2): 190-197 (2000)	Liu et al.
275.	Patterning cells and their environments using multiple laminar fluid flows in capillary networks, <i>Proc. Natl. Acad. Sci. USA</i> , 96: 5545-5548 (1999)	Takayama et al.

Description / Title		Author / Inventor
276.	Perfectly monodisperse microbubbling by capillary flow focusing: An alternate physical description and universal scaling, <i>Physical Review</i> , 69: 027301-1 – 027301-3 (2004)	Gañán-Calvo
277.	Perfluoroalkylated amphiphiles with a morpholinophosphate or a dimorpholinophosphate polar head group, <i>New J. Chem</i> , 609-613 (1998) (“Sadtler”)	Sadtler et al.
278.	Perfluorocarbon Fluids, in <i>Organofluorine, Chemistry</i> , 89-119 (1994) (“Green”)	Green et al.
279.	Perfluorochemicals: their applications and benefits to cell culture, <i>Tibtech</i> , 16: 272-277 (1998)	Lowe et al.
280.	Perry’s Chemical Engineer’s Handbook, 3-247 to -252, 3-280 to -282, Don W. Green ed., 6th ed. (1984)	
281.	Pierce Catalog and Handbook	
282.	Preparation of Monodispersed Solid Lipid Microspheres Using a Microchannel Emulsification Technique, <i>Journal of Colloid and Interface Science</i> , 227: 95-103 (2000)	Sugiura et al.
283.	Preparation of Titania Particles Utilizing the Insoluble Phase Interface in a Microchannel Reactor, <i>Chemical Communications</i> , 1462-1463 (2002)	Wang et al.
284.	Production and Characterization of Monodispersed Oil-in-Water Microspheres Using Microchannels, <i>Food Sci. Technol. Res.</i> , 5(4): 350-355 (1999)	Kobayashi et al.
285.	Protein crystallization by capillary counter-diffusion for applied crystallographic structure determination, <i>J. Struct. Bio.</i> , 142: 218-231 (2003)	Ng et al.
286.	Quantitation of Targets for PCR by Use of Limiting Dilution, <i>BioTechnologies</i> , 13(3) (1992)	Sykes et al.
287.	Quantitative detection of protein expression in single cells using droplet microfluidics, <i>Chern. Commun</i> , 1218-1220 (2007)	Huebner et al.

Description / Title		Author / Inventor
288.	Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane), <i>Analytical Chemistry</i> , 70: 4974-4984 (1998)	Duff et al.
289.	Real time quantitative PCR, <i>Genome Res.</i> , 6: 986-994 (1996)	Heid et al.
290.	Refractive index matching methods for liquid flow investigations, Experiments in Fluids; Experimental Methods and their Applications to Fluid flow, <i>Springer International</i> , Volume 17(5) (1994)	Budwig
291.	Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae <i>Chlorella vulgaris</i> coimmobilized in alginate beads with the microalgae growth-promoting bacterium <i>Azospirillum brasilense</i> , <i>Water Research</i> , 36: 2941-2948 (2002)	De-Bashan et al.
292.	Resorufin Safety Data Sheet, Revision 3.00; Modified 2013-10-25	
293.	Rise of Liquids and Bubbles in Angular Capillary tubes, <i>Journal of colloid and Interface Science</i> , 247: 162-166	Bico et al.
294.	Screening of Protein Crystallization Conditions on a Microfluidic Chip Using Nanoliter-Size Droplets, <i>Journal of the American Chemical Society</i> , 125(37): 11170-11171 (2003)	Zheng et al.
295.	Selective Encapsulation of Single Cells and Subcellular Organelles into Picaliter- and Femtoliter-Volume Droplets, <i>Analytical Chemistry</i> , 77(6):1539-1544 (2005)	He et al.
296.	Self-Propelling Slugs, <i>J. Fluid Mech.</i> , 467: 101-127 (2002)	Bico et al.
297.	Shear Rupturing of Droplets in Complex Fluids, <i>Langmuir</i> , 13, 4600-4613 (1997) ("Mason")	Mason
298.	Shimadzu Corporation History, as accessed on April 5, 2016, from shimadzu.com/about/history.html	

Description / Title		Author / Inventor
299.	Simultaneous generation of droplets with different dimensions in parallel integrated microfluidic droplet generators, <i>Soft Matter</i> , 4: 258-262 (2008)	Li et al.
300.	Simultaneous Rapid Measurement of Whole Blood Myoglobin, Creatine Kinase MB, and Cardiac Troponin I by the Triage Cardiac Panel for Detection of Myocardial Infarction, <i>Clin. Chem.</i> , 45:199- 205 (1999)	Apple et al.
301.	Single-cell analysis by RT-PCR reveals differential expression of multiple type 1 and 2 cytokine genes among cells within polarized CD4+ T cell populations,” <i>International Immunology</i> 11(4): 617-621 (1999)	Kelso et al.
302.	Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device, <i>Anal. Chem.</i> , 73:565-570 (2001) (“Lagally”)	Lagally et al.
303.	Size-Controlled Growth of CdSe Nanocrystals in Microfluidic Reactors, <i>Nano Letters</i> , 3(2): 199-201 (2003)	Chan et al.
304.	Splitting a Liquid Droplet for Electrowetting-Based Microfluidics, Proceedings of 2001 ASME International Mechanical Engineering Congress and Exposition, 1-7 (2001)	Cho et al.
305.	Stability Criteria for Emulsions, <i>Physical Review Letters</i> , 69(16): 19 (1992)	Bibette et al.
306.	Stability of emulsions under equilibrium and dynamic conditions, Colloids and Surfaces a: Physicochemical and Engineering Aspects, Elsevier Science B.V. (1987)	Ivanov et al.
307.	Successes and challenges of lab-on-a-chip, <i>Lab Chip</i> , 2: 42N -47N (2002)	Kopf-Sill
308.	Surface Logix: Mini Disease Models for Maximum Impact, <i>Chemistry & Biology</i> , 10: 289-291 (2003)	Mountain
309.	Surfactant Effect on Production of Monodispersed Microspheres by Microchannel Emulsification Method, <i>Journal of Surfactants and Detergents</i> , 3(3) (2000)	Tong et al.

Description / Title		Author / Inventor
310.	Surfactants and Subsurface Remediation, <i>Envtl. Sci. Tech.</i> , 26: 2324 (1992)	West et al.
311.	Synthesis of Polymeric microspheres with Narrow Size Distributions Employing Microchannel Emulsification, <i>Macromol. Rapid Commun.</i> , 22: 773-778 (2001)	Sugiura et al.
312.	Synthesis of zinc oxide nanoparticles with controlled morphology, Royal Society of Chemistry (1999)	Wang et al.
313.	Table of Contents to the Micro Total Analysis Systems 1994: Proceedings of the μ TAS 1994 Symposium	
314.	Table of Contents to the Micro Total Analysis Systems 1996: Proceedings of the μ TAS 1996 Symposium	
315.	Table of Contents to the Micro Total Analysis Systems 1998: Proceedings of the μ TAS 1998 Symposium	
316.	Table of Contents to the Micro Total Analysis Systems 2000: Proceedings of the μ TAS 2000 Symposium	
317.	Table of Contents to the Micro Total Analysis Systems 2001: Proceedings of the μ TAS 2001 Symposium	
318.	Textbook of polymer Science, Third Edition, John Wiley & Sons, Inc. (1984)	Billmeyer
319.	The burgeoning power of the shrinking laboratory, <i>Nature Biotechnology</i> , 17: 1061-1062 (1999)	Ramsey
320.	The deformation and breakup of liquid drops in low Reynolds number flow through a capillary, <i>Phys. Fluids A</i> , 4(7) (July 1992)	Olbricht et al.
321.	The effect of the hydrophobicity of microchannels and components in water and oil phases on droplet formation in microchannel water-in-oil emulsification, <i>Colloids and Surfaces A: Physicochem. Eng. Aspects</i> , 179: 29-37 (2001)	Kawakatsu et al.

Description / Title		Author / Inventor
322.	The examination of hydrocarbon oils and of saponifiable fats and waxes, 200 (1922)	Holde
323.	The History of the International Symposium on Microscale Separations and Bioanalysis, as accessed on April 5, 2016, from http://www.msb2016.org/about/about/	
324.	The intensification of rapid reactions in multiphase systems using slug flow in capillaries, <i>Lab on a Chip</i> , 1: 10-15 (2001) (“Burns (2001)”)	Burns et al.
325.	The next chip-based Revolution, <i>Chemistry & Biology</i> , 5: R115–116 (1998)	Wells
326.	Top 10 Innovations 2014, THE SCIENTIST, (December 1, 2014) available at http://www.the-scientist.com/?articles.view/articleNo/41486/title/Top-10-Innovations-2014/ , last accessed May 1, 2015	
327.	Toxicological Profile for Nitrophenols, Agency for Toxic Substances and Disease Registry U.S. Public Health Service	
328.	Transport of gas bubbles in capillaries, <i>Phys. Fluids A</i> , 1(10): 1642-1655 (1989)	Ratulowski et al.
329.	Ultrahydrophobic Surfaces. Effects of Topography Length Scales on Wettability, <i>Langmuir</i> , 16: 7777-7782 (2000)	On�r et al.
330.	Vitae LLC Company information, as accessed on April 5, 2016, from http://www.vitaellc.com/about.htm	
331.	Towards Integrated Continuous-Flow Chemical Reactors, <i>Mikrochim. Acta</i> , 131: 19-24 (1999) (“Erbacher”)	Erbacher et al.
332.	Rapid Microfluidic Mixing, <i>Anal. Chem.</i> , 74:45-51 (2002) (“Johnson”)	Johnson et al.
333.	Ordered and Disordered Patterns in Two-Phase Flows in Microchannels, <i>Phys. Rev. Lett.</i> , 90(14): 144505 (2003)	Dreyfus et al.
334.	Capillary Electrophoresis on a Microchip, <i>Electrophoresis</i> , 21: 41-54 (2000)	Dolnik et al.

Description / Title		Author / Inventor
335.	Soft Lithography in Biology and Biochemistry, <i>Annu. Rev. Biomed. Eng.</i> , 3: 335–73 (2001)	Whitesides et al.
336.	Chip technology for micro-separation, <i>BioMethods</i> , Vol. 10 (1999)	de Mello et al.
337.	Toward effective PCR-based amplification of DNA on microfabricated chips, <i>Capillary Electrophoresis of Nucleic Acids</i> , 2: 191-204, (2001)	Ferrance et al.
338.	Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device	Woolley et al.
339.	Effect of highly fragmented DNA on PCR, <i>Nucleic Acids Research</i> , 24: 5026–5033 (1996)	Golenberg et al.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
A method for conducting a reaction in plugs in a microfluidic system, comprising the steps of:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0020]; Ex. 1 at 8:16-20. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0119]; Ex. 1 at 37:5-6. • Ex. 2 at [0095]. • Ex. 2 at [0296].
providing the microfluidic system comprising at least two channels having at least one junction;	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-19. • Ex. 2 at [0015]; Ex. 1 at 6:17-19. • Ex. 2 at [0065]; Ex. 1 at 19:7-8. • Ex. 2 at [0068]; Ex. 1 at 19:23-27. • Ex. 2 at [0070]; Ex. 1 at 20:15-18. • Ex. 2 at [0084]; Ex. 1 at 25:7-14. • Ex. 2 at [0125]; Ex. 1 at 25:13-16. • Ex. 2 at [0292]; Ex. 1 at 83:8-16. • Ex. 2 at [0323]; Ex. 1 at 86: 14-16. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A.
continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels;	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0012]; Ex. 1 at 5:17-23. • Ex. 2 at [0014]; Ex. 1 at 6:3-5. • Ex. 2 at [0020]; Ex. 1 at 8:14-18.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0113]; Ex. 1 at 34:23-35:2. • Ex. 2 at [0116]; Ex. 1 at 35:19-23. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0166]; Ex. 1 at 50:7-13. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4.
continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels;	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0020]; Ex. 1 at 8:14-18. • Ex. 2 at [0022]; Ex. 1 at 9:14-16. • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0070]; Ex. 1 at 20:15-21. • Ex. 2 at [0113]; Ex. 1 at 34:26-29. • Ex. 2 at [0116]; Ex. 1 at 35:19-27. • Ex. 2 at [0125]; Ex. 1. at 38:16-19. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0290]; Ex. 1 at 82:22-27. • Ex. 2 at [0301]. • Ex. 2 at [0323].
forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
<p>flowing immiscible carrier fluid at the junction of the at least two channels,</p>	<ul style="list-style-type: none"> • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0015]; Ex. 1 at 7:7-10. • Ex. 2 at [0020]; Ex. 1 at 8:18-27. • Ex. 2 at [0070]; Ex. 1 at 20:15-21. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [0125]; Ex. 1 at 38:13-16. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A.
<p>the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel,</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0100]; Ex. 1 at 30:2-3. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [00241]; Ex. 1 at 70:22-25. • Ex. 2 at [0041]. • Ex. 2 at [0042]. • Ex. 2 at Figs. 19J and 19L.
<p>wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule; and</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0014]; Ex. 1 at 6:10-14. • Ex. 2 at [0020]; Ex. 1 at 8:14-21. • Ex. 2 at [0058]; Ex. 1 at 15:18-19. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0106]; Ex. 1 at 32:14-16. • Ex. 2 at [0113]; Ex. 1 at 34:23-35:2.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0323].
providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0012]; Ex. 1 at 5:17-23. Ex. 2 at [0020]; Ex. 1, at 8:14-21. Ex. 2 at [0058]; Ex. 1 at 15:18-19. Ex. 2 at [0078]; Ex. 1 at 23:12-20. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0106]; Ex. 1 at 32:14-16. Ex. 2 at [0113]; Ex. 1 at 34:23-35:2. Ex. 2 at [0120]; Ex. 1 at 37:12-19. Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0296].
Claim 2	Prior Art
The method according to claim 1, wherein the at least one biological molecule is DNA or RNA.	<p><i>See claim 1, above.</i></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0020]; Ex. 1 at 8:18-21. Ex. 2 at [0021]; Ex. 1 at 9:3-7. Ex. 2 at [0050]; Ex. 1 at 13:18-19. Ex. 2 at [0052]; Ex. 1 at 13:23-29. Ex. 2 at [0058]; Ex. 1 at 15:18-16:3. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0108]; Ex. 1 at 33:12-14. Ex. 2 at [0109]; Ex. 1 at 33:28-34:2.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 2	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at [0113]; Ex. 1 at 34:23-26. Ex. 2 at [0166]; Ex. 1 at 50:7-13.
Claim 3	Prior Art
The method according to claim 2, wherein the reaction is an autocatalytic reaction.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0080]; Ex. 1 at 1 24:12-13.
Claim 4	Prior Art
The method according to claim 2, wherein the reaction is a polymerase chain reaction.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0080]; Ex. 1 at 24:12-13.
Claim 5	Prior Art
The method according to claim 1, wherein the reaction is an enzymatic reaction.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0021]; Ex. 1 at 9:3-7. Ex. 2 at [0058]; Ex. 1 at 15:29-16:2. Ex. 2 at [0078]; Ex. 1 at 23:16-20. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0099]; Ex. 1 at 29:8-10.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 5	Prior Art
	<ul style="list-style-type: none"> • Ex. 2 at [0106]; Ex. 1 at 32:14-16. • Ex. 2 at [0108]; Ex. 1 at 33:12-14. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0045].
Claim 8	Prior Art
<p>The method according to claim 1, wherein the immiscible carrier fluid is an oil.</p>	<p>See claim 1, above.</p> <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0020]; Ex. 1 at 8:27-28. • Ex. 2 at [0022]; Ex. 1 at 9:18-20. • Ex. 2 at [0100]; Ex. 1 at 30:2-3. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0287]; Ex. 1 at 82:2-6. • Ex. 2 at [0290]; Ex. 1 at 82:22-27. • Ex. 2 at [0323]; Ex. 1 at 86:14-17. • Ex. 2 at [0096]. • Ex. 2 at [0300].

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 9	Prior Art
<p>The method according to claim 8, wherein the oil comprises a surfactant.</p>	<p>See claim 8, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0022]; Ex. 1 at 9:18-20. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0094]; Ex. 1 at 28:21-23. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. • Ex. 2 at [0096]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0020]. • Ex. 2 at [0300].
Claim 10	Prior Art
<p>The method according to claim 9, wherein the surfactant is a fluorosurfactant.</p>	<p>See claim 9, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1.
Claim 11	Prior Art
<p>The method according to claim 8, wherein the oil is a fluorinated oil.</p>	<p>See claim 8, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0118]; Ex. 1 at 36:8-10.

APPENDIX B – U.S. Patent No. 8,329,407 (Anticipation)

Claim 13	Prior Art
The method according to claim 1, wherein the providing step includes heating.	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0080]; Ex. 1 at 24:12-13.

APPENDIX C – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
A method for conducting a reaction in plugs in a microfluidic system, comprising the steps of:	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 4. • Ex. 16 at Fig. 1. • Ex. 39 at 3:82-86.
providing the microfluidic system comprising at least two channels having at least one junction;	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-101. • Ex. 16 at 4. • Ex. 16 at Fig. 1; Ex. 39 at Figs. 1-5 and 9.
continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels;	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. • Ex. 39 at 3:4-6. • Ex. 39 at 4:30-33. • Ex. 39 at Fig. 1.
continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels;	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 4-5. • Ex. 16 at 12. • Ex. 16 at Abstract. • Ex. 39 at Fig. 1. • Ex. 39 at 3:82-86.

APPENDIX C – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 39 at 3:102-104.
forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels,	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12 Ex. 16 at Abstract.
the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel,	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. Ex. 39 at Fig. 1. Ex. 39 at 3:102-104.
wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule; and	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 3:70-86.
providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 2:44-50. Ex. 39 at 3:57-60. Ex. 39 at 3:70-73.

APPENDIX C – U.S. Patent No. 8,329,407 (Anticipation)

Claim 2	Prior Art
<p>The method according to claim 1, wherein the at least one biological molecule is DNA or RNA.</p>	<p>See claim 1, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:70-86. • Ex. 39 at 3:82-86. • Ex. 39 at 4:30-33.
Claim 5	Prior Art
<p>The method according to claim 1, wherein the reaction is an enzymatic reaction.</p>	<p>See claim 1, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:82-86.
Claim 8	Prior Art
<p>The method according to claim 1, wherein the immiscible carrier fluid is an oil.</p>	<p>See claim 1, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:41.

APPENDIX C – U.S. Patent No. 8,329,407 (Anticipation)

<p>Claim 9</p> <p>The method according to claim 8, wherein the oil comprises a surfactant.</p>	<p>Prior Art</p> <p>See claim 8, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-48. • Ex. 39 at 2:19-26. • Ex. 39 at 4:26-29.
<p>Claim 10</p> <p>The method according to claim 9, wherein the surfactant is a fluorosurfactant.</p>	<p>Prior Art</p> <p>See claim 9, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 1:39-41.
<p>Claim 11</p> <p>The method according to claim 8, wherein the oil is a fluorinated oil.</p>	<p>Prior Art</p> <p>See claim 8, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:39-41.

APPENDIX C – U.S. Patent No. 8,329,407 (Anticipation)

Claim 13	Prior Art
The method according to claim 1, wherein the providing step includes heating.	<p>See claim 1, above.</p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 5; Ex 39 at 2:44-52.• Ex. 39 at 3:57-60.• Ex. 39 at 3:70-73.

APPENDIX D – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
A method for conducting a reaction in plugs in a microfluidic system, comprising the steps of:	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 20. • Id. at 36.
providing the microfluidic system comprising at least two channels having at least one junction;	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 18. • Id. at 19. • Id. at 25. • Id. at 53. • Id. at 56. • Id. at 57.
continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at least two channels;	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 20. • Id. at 62. • Id. at 133. • Id. at 135. • Id. at 147.
continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17.

APPENDIX D – U.S. Patent No. 8,329,407 (Anticipation)

Claim 1	Prior Art
two channels;	<ul style="list-style-type: none"> • Id. at 18. • Id. at 61.
forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels,	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 19. • Id. ta 62. • Id. at 133. • Id. at 147.
the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel,	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 19. • Id. at 62. • Id. at 149.
wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule; and	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 133. • Id. at 147-8.
providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 147-8.

APPENDIX D – U.S. Patent No. 8,329,407 (Anticipation)

Claim 2	Prior Art
<p>The method according to claim 1, wherein the at least one biological molecule is DNA or RNA.</p>	<p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 44 at 135. Id. at 147-8. <p><u>Shaw Stewart Thesis</u></p>
Claim 5	Prior Art
<p>The method according to claim 1, wherein the reaction is an enzymatic reaction.</p>	<p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 43. Id. at 89. Id. at 135. Id. at 147-8. <p><u>Shaw Stewart Thesis</u></p>
Claim 8	Prior Art
<p>The method according to claim 1, wherein the immiscible carrier fluid is an oil.</p>	<p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 44 at 18. Id. at 45. Id. at 66-7. <p><u>Shaw Stewart Thesis</u></p>

APPENDIX D – U.S. Patent No. 8,329,407 (Anticipation)

Claim 8		Prior Art	<ul style="list-style-type: none"> Id. at 89.
Claim 10	The method according to claim 9, wherein the surfactant is a fluorosurfactant.	Prior Art	<p>See claim 9, above.</p> <ul style="list-style-type: none"> Ex. 44 at 18. Id. at 89. <p><u>Shaw Stewart Thesis</u></p>
Claim 11	The method according to claim 8, wherein the oil is a fluorinated oil.	Prior Art	<p>See claim 8, above.</p> <ul style="list-style-type: none"> Ex. 44 at 18. <p><u>Shaw Stewart Thesis</u></p>
Claim 13	The method according to claim 1, wherein the providing step includes heating.	Prior Art	<p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 44 at 147-8. <p><u>Shaw Stewart Thesis</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
A method of conducting a reaction within at least one plug comprising the steps of:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0012]; Ex. 1 at 5:15-23. • Ex. 2 at [0020]; Ex. 1 at 8:18-20.
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0084]-[0085].
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 7:58-68. • Id. at Fig. 1. • Id. at Fig. 2. • Id. at 3:20-37. • Id. at 3:65-4:22. • Id. at 7:58-68.
	<p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> • Ex. 23 at 33:6-19. • Id. at 33:6-34:2. • Id. at Fig. 1C. • Id. at Fig. 2. • Id. at Fig. 3.
	<p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none"> • Ex. 25 at 5:15-19.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 12:60-13:2. • Id. at 14:3-19. • Id. at Fig. 5B. <p style="text-align: center;"><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-26. • Id. at 3:25-29. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Introduction. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
introducing a carrier-fluid into a first microchannel of a device;	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:17-25. • Ex. 2 at Abstract; Ex. 1 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at [0014]; Ex. 1 at 6:14-16. Ex. 2 at [0287]; Ex. 1 at 82:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 3:65-4:22. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> Ex. 28 at 2:12-26. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
<p>simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the streams contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug;</p>	<p><u>Quake (US 2002/058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0018]; Ex. 1 at 7:20-8:5. • Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. • Ex. 2 at [0318]; Ex. 1 at 85:20-25. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at Fig. 22; Ex. 1 at Fig. 18. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<p><u>Kenis (1999)</u></p> <ul style="list-style-type: none"> • Ex. 37 at 83:3. • Id. at Fig. 2. • Id. at 83:2. <p><u>Johnson (2000)</u></p> <ul style="list-style-type: none"> • Ex. 45 at Abstract. • Id. at 46-48. • Id. at 51. <p><u>Erbacher (1999)</u></p> <ul style="list-style-type: none"> • Ex. 46 at Abstract. • Id. at 19-20. • Id. at 22-24. <p><u>Whitesides (2000)</u></p> <ul style="list-style-type: none"> • Ex. 54 at 942. • Id. at 944-946. • Id. at Fig. 1. <p><u>Weigl (1999)</u></p> <ul style="list-style-type: none"> • Ex. 32 at 346. • Id. at Fig. 1. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 9D. • Id. at [0084]-[0085]. • Id. at [0103]. • Id. at [0105]. • Id. at [0138]-[0139]. • Id. at [0142]-[0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 7:58-68. • Id. at Fig. 1. • Id. at Fig. 2. • Id. at 3:20-37. • Id. at 3:65-4:22. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> • Ex. 23 at 33:6-34-19. • Id. at 33:20-34:2. • Id. at Fig. 1C. • Id. at Fig 2. • Id. at Fig. 3. <p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none"> • Ex. 25 at 5:15-19. • Id. at 12:60-13:2. • Id. at 14:3-19. • Id. at Fig. 5B. <p><u>Wang (WO 00/23181)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 28 at 2:12-26. Id. at 3:25-29. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018]. <p><u>Floyd (2002)</u></p> <ul style="list-style-type: none"> Ex. 40 at 92-93.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
each plug is substantially surrounded by carrier.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0105]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 6:60-63. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 7 at Abstract.• Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 10-11.• Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none">• Ex. 38 at [0018].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 2	Prior Art
<p>The method of claim 1, wherein the carrier-fluid comprises an oil.</p>	<p>See claim 1, above.</p> <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:12-17. • Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 2	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 9:35-40. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 36.
Claim 3	Prior Art
<p>The method of claim 1, wherein the carrier-fluid comprises a fluorinated compound.</p>	<p><i>See claim 1, above.</i></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0116]-[0117]; Ex. 1 at 35:25-36:1. Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 4; Ex. 39 at 1:41. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> Ex. 14 at 3:62-66. Id. at 6:36-50. Id. at 8:45-9:19. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0047].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 3	Prior Art
	<p data-bbox="280 575 313 934"><u>Paolini (US 2002/0131147)</u></p> <ul data-bbox="350 989 459 1255" style="list-style-type: none"> • Ex. 19 at [0075]. • Id. at [0076]. • Id. at [0077]. <p data-bbox="496 552 529 957"><u>Shenderov (US 2002/0043463)</u></p> <ul data-bbox="568 989 600 1255" style="list-style-type: none"> • Ex. 20 at [0033]. <p data-bbox="638 606 670 903"><u>Wang (WO 00/23181)</u></p> <ul data-bbox="708 976 740 1255" style="list-style-type: none"> • Ex. 28 at 3:26-28. <p data-bbox="777 661 810 848"><u>Curcio (2002)</u></p> <ul data-bbox="847 1043 880 1255" style="list-style-type: none"> • Ex. 33 at 36. <p data-bbox="899 604 932 913"><u>Krafft (U.S. 5,980,936)</u></p> <ul data-bbox="974 972 1196 1255" style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23. <p data-bbox="1240 571 1273 877"><u>Parris (U.S. 5,739,036)</u></p> <ul data-bbox="1317 972 1425 1255" style="list-style-type: none"> • Ex. 42 at Abstract. • Ex. 42 at 2:29-34. • Ex. 42 at 3:16-23.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 5	Prior Art
<p>The method of claim 1, wherein the carrier-fluid comprises at least one surfactant.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0020]. • Id. at [0096]. • Id. at [0116]-[0117]; Ex. 1 at 35:25-36:1. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-47. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]-[0077]. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 5	Prior Art
	<p data-bbox="280 651 313 867"><u>Schubert (1993)</u></p> <ul data-bbox="332 972 365 1255" style="list-style-type: none"> • Ex. 31 at Abstract. <p data-bbox="384 661 417 856"><u>Sadtler (1998)</u></p> <ul data-bbox="453 972 485 1255" style="list-style-type: none"> • Ex. 34 at Abstract. <p data-bbox="505 611 537 919"><u>Krafft (U.S. 5,980,936)</u></p> <ul data-bbox="573 972 808 1255" style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23.
Claim 6	Prior Art
<p data-bbox="956 1392 1057 1894">The method of claim 1, wherein the at least one of the plug-fluids comprises a solvent.</p>	<p data-bbox="956 1071 989 1316">See claim 1, above.</p> <p data-bbox="1024 474 1057 1037"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul data-bbox="1092 785 1125 1255" style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:12-17. <p data-bbox="1161 453 1193 1058"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul data-bbox="1218 812 1328 1255" style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 6	<div> <div>Prior Art</div> <div> <div><u>Nisisako Abstract (2001)</u></div> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <div><u>Thorsen (2001)</u></div> <ul style="list-style-type: none"> Ex. 13 at 4163. <div><u>Nisisako (2002)</u></div> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <div><u>Burns (2001)</u></div> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. </div> </div>
Claim 11	<div> <div>Prior Art</div> <div> <div>See claim 1, above.</div> <div><u>Quake (US 2002/0058332; US 60/233,037)</u></div> <ul style="list-style-type: none"> Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0316]-[0317]; Ex. 1 at 84:28-85:10. Ex. 2 at [0316]-[0317]; Ex. 1 at 85:12-19. </div> </div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 11	Prior Art
	<p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> Ex. 28 at 2:12-17. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> Ex. 23 at 33:6-19. Id. at 33:20-34:2. Id. at Fig 1C. Id. at Fig. 2. Id. at Fig. 3. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 7:63-68. Id. at 3:20-37. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> Ex. 26 at 652. Id. at 655. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0139]-[0140].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 23	Prior Art
<p>The method of claim 1, further comprising detecting the product of the reaction.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 6-7; Ex. 39 at 2:94-105. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 11. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 4:19-22. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0143].
Claim 27	Prior Art
<p>The method of claim 1, wherein refractive indices of the carrier-fluid and the plug-fluids are substantially similar.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0173]; Ex. 1 at 53:1-4.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 27	Prior Art
	<p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none">• Ex. 14 at 6:36-50. <p><u>Green (1994)</u></p> <ul style="list-style-type: none">• Ex. 30 at 4, Table 1.• Id. at 114.• Id. at 94, Table 3.
Claim 29	Prior Art
The method of claim 1, further comprising employing a number of devices in parallel.	<p><i>See claim 1, above.</i></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0067]; Ex. 1 at 19:17-22.• Ex. 2 at [0084]; Ex. 1 at 25:7-11.• Ex. 2 at [0214]; Ex. 1 at 62:20-21.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 31	Prior Art
<p>The method of claim 1, wherein the reaction is a polymerization reaction.</p>	<p>See claim 1, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0012]. • Id. at [0013]-[0014]. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 89-90. • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 31	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 7:58-68. • Id. at Fig. 1. • Id. at Fig. 2. • Id. at 3:20-37. • Id. at 3:65-4:22. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655 <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019.
Claim 33	Prior Art
The method of claim 1, wherein each plug initially has a cross section that is substantially the same size as the cross	See claim 1, above.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 33	Prior Art
section of the channel at the inlet.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0091]-[0092]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0025].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 35	Prior Art
The method of claim 1, wherein the volume of at least one plug is about 1 femtoliter to about 250 nL.	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0003]; Ex. 1 at 2:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Abstract.• Id. at Experimental.• Id. at Fig. 1.• Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 10-11.• Id. at 14.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 35	Prior Art
	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0025].
Claim 36	Prior Art
A method of conducting a reaction within at least one plug comprising the steps of:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0012]; Ex. 1 at 5:15-23. Ex. 2 at [0020]; Ex. 1 at 8:18-20. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 7:58-68. Id. at 3:20-37. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> Ex. 23 at 33:6-33:19. Id. at Fig. 1C. Id. at Fig. 2. Id. at Fig. 3. Id. at 33:20-34:2.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<div> <div> <ul style="list-style-type: none"> Ex. 25 at 5:11-15. </div> <div> <ul style="list-style-type: none"> Ex. 28 at 2:12-17. </div> <div> <ul style="list-style-type: none"> Ex. 17 at Introduction. </div> <div> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at 26. </div> <div> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. </div> <div> <ul style="list-style-type: none"> Ex. 38 at [0139]. </div> </div> <div> <div> <p><u>Kopf-Sill (US 5,842,787)</u></p> </div> <div> <p><u>Wang (WO 00/23181)</u></p> </div> <div> <p><u>Nisisako Abstract (2001)</u></p> </div> <div> <p><u>Nisisako (2002)</u></p> </div> <div> <p><u>Burns (2001)</u></p> </div> <div> <p><u>Seki (US 2002/0195463)</u></p> </div> </div>
introducing a carrier-fluid into a first microchannel of a device;	<div> <ul style="list-style-type: none"> Ex. 2 at [0015]; Ex. 1 at 6:17-25. Ex. 2 at Abstract; Ex. 1 at Abstract. Ex. 2 at [0014]; Ex. 1 at 6:14-16. Ex. 2 at [0287]; Ex. 1 at 82:2-3. </div> <div> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> </div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.• Ex. 16 at 12.• Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at Fig. 9D.• Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at 7:58-68.• Id. at 3:20-37. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none">• Ex. 28 at 2:12-26. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Id. at 25. • Ex. 35 at 10-11. • Ex. 38 at [0018]. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p>
<p>simultaneously introducing at least two streams of plug-fluids into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction of the first inlet and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent different from the first reagent; each plug-fluid is immiscible with the carrier-fluid; and each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug;</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0018]; Ex. 1 at 7:20-8:5. • Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. • Ex. 2 at [0318]; Ex. 1 at 85:20-25. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at Fig. 22; Ex. 1 at Fig. 18. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p style="text-align: center;"><u>Kenis (1999)</u></p> <ul style="list-style-type: none"> • Ex. 37 at 83:3. • Id. at Fig. 2. • Id. at 83:2.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<p><u>Johnson (2000)</u></p> <ul style="list-style-type: none"> • Ex. 45 at Abstract. • Id. at 46-48. • Id. at 51. <p><u>Erbacher (1999)</u></p> <ul style="list-style-type: none"> • Ex. 46 at Abstract. • Id. at 19-20. • Id. at 22-24. <p><u>Whitesides (2000)</u></p> <ul style="list-style-type: none"> • Ex. 54 at 942. • Id. at 944-946. • Id. at Fig. 1. <p><u>Weigl (1999)</u></p> <ul style="list-style-type: none"> • Ex. 32 at 346. • Id. at Fig. 1. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0084]-[0085]. • Id. at [0103]. • Id. at [0105]. • Id. at [0138]-[0139].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Id. at [0142]-[0144]. • Ex. 6 at 7:58-68. • Id. at 3:20-37. • Id. at 3:65-4:22. <p><u>Chow (WO 01/02850 A1)</u></p> <ul style="list-style-type: none"> • Ex. 23 at 33:6-34:2. • Id. at Fig. 1C. • Id. at Fig. 2. • Id. at Fig. 3. <p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none"> • Ex. 25 at 5:15-19. • Id. at 12:60-13:2. • Id. at 14:3-19. • Id. at Fig. 5B. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-26. • Id. at 3:25-29. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<ul style="list-style-type: none"> Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018]. <p><u>Floyd (2002)</u></p> <ul style="list-style-type: none"> Ex. 40 at 92-93.
and each plug is substantially surrounded by carrier.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<div> <ul style="list-style-type: none"> Ex. 16 at Abstract. </div> <div> <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0105]. </div> <div> <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 6:60-63. </div> <div> <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. </div> <div> <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. </div> <div> <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. </div> <div> <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. </div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 36	Prior Art
	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018].
Claim 37	Prior Art
A method of conducting a reaction within at least one plug comprising the steps of:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0012]; Ex. 1 at 5:15-23. Ex. 2 at [0020]; Ex. 1 at 8:18-20. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 7:58-68. Id. at 3:20-37. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> Ex. 23 at 33:6-34:2. Id. at Fig. 1C. Id. at Fig. 2. Id. at Fig. 3. <p><u>Kopf-Sill (US 5,842,787)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<ul style="list-style-type: none"> • Ex. 25 at 5:11-15. <p style="text-align: center;"><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-17. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Introduction. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
introducing a carrier-fluid into a first microchannel of a device	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:17-25. • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at 9D. Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 7:58-68. Id. at 3:20-37. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> Ex. 28 at 2:12-26. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<ul style="list-style-type: none"> • Id. at 25. • Ex. 35 at 10-11. • Id. at 14. • Ex. 38 at [0018]. <p style="text-align: center;"><u>Burns (2001)</u></p> <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p>
<p>introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the first and second plug-fluids contact the carrier-fluid; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug;</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0018]; Ex. 1 at 7:20-8:5. • Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. • Ex. 2 at [0318]; Ex. 1 at 85:20-25. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at Fig. 22; Ex. 1 at Fig. 18. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p style="text-align: center;"><u>Kenis (1999)</u></p> <ul style="list-style-type: none"> • Ex. 37 at 83:3. • Id. at Fig. 2.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<div> <ul style="list-style-type: none"> Id. at 83:2. </div> <div> <p><u>Johnson (2000)</u></p> <ul style="list-style-type: none"> Ex. 45 at Abstract. Id. at 46-48. Id. at 51. </div> <div> <p><u>Erbacher (1999)</u></p> <ul style="list-style-type: none"> Ex. 46 at Abstract. Id. at 19-20. Id. at 22-24. </div> <div> <p><u>Whitesides (2000)</u></p> <ul style="list-style-type: none"> Ex. 54 at 942. Id. at 944-946. Id. at Fig. 1. </div> <div> <p><u>Weigl (1999)</u></p> <ul style="list-style-type: none"> Ex. 32 at 346. Id. at Fig. 1. </div> <div> <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0084]-[0085]. Id. at [0103]. Id. at [0105]. </div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Id. at [0138]-[0139].• Id. at [0142]-[0144].• Ex. 6 at 7:58-68.• Id. at Fig. 1.• Id. at Fig. 2.• Id. at 3:20-37.• Id. at 3:65-4:22. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none">• Ex. 23 at 33:6-34:2.• Id. at Fig. 1C.• Id. at Fig. 2.• Id. at Fig. 3. <p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none">• Ex. 25 at 5:15-19.• Id. at 12:60-13:2.• Id. at 14:3-19.• Id. at Fig. 5B. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none">• Ex. 28 at 2:12-26.• Id. at 3:25-29.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<p data-bbox="332 598 365 934"><u>Nisisako Abstract (2001)</u></p> <ul data-bbox="397 840 552 1270" style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p data-bbox="584 661 617 871"><u>Thorsen (2001)</u></p> <ul data-bbox="657 1018 690 1270" style="list-style-type: none"> • Ex. 13 at 4163. <p data-bbox="722 661 755 871"><u>Nisisako (2002)</u></p> <ul data-bbox="795 997 868 1270" style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p data-bbox="901 672 933 861"><u>Burns (2001)</u></p> <ul data-bbox="974 1008 1047 1270" style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p data-bbox="1063 609 1096 934"><u>Seki (US 2002/0195463)</u></p> <ul data-bbox="1120 997 1153 1270" style="list-style-type: none"> • Ex. 38 at [0018]. <p data-bbox="1169 682 1201 861"><u>Floyd (2002)</u></p> <ul data-bbox="1226 1008 1258 1270" style="list-style-type: none"> • Ex. 40 at 92-93.
and each plug is substantially surrounded by carrier.	<p data-bbox="1299 483 1331 1050"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul data-bbox="1364 819 1396 1270" style="list-style-type: none"> • Ex. 2 at [0100]; Ex 1 at 32:2-3.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.• Ex. 16 at 12.• Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at Fig. 9D.• Id. at [0105]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at 6:60-63. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Abstract.• Id. at Experimental.• Id. at Fig. 1.• Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract.• Id. at Fig. 1.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 37	Prior Art
	<div><div><div><div>• Ex. 35 at 10-11.</div><div>• Id. at 14.</div><div>• Ex. 38 at [0018].</div></div><div><div><u>Burns (2001)</u></div><div><u>Seki (US 2002/0195463)</u></div></div></div></div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 38	Prior Art
<p>The method of claim 37, wherein the carrier-fluid comprises an oil.</p>	<p>See claim 37, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:12-17. • Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4-5. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:62-66. • Id. at 6:36-50. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p><u>Thorsen (1999)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Nisisako (2002)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 38	Prior Art
	<ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at 25. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0047]. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 9:35-40. <p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> Ex. 42 at Abstract. Ex. 42 at 2:29-34. Ex. 42 at 3:16-23.
Claim 39	Prior Art
The method of claim 37, wherein the carrier-fluid comprises at least one surfactant.	<p>See claim 37, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0020]; Ex. 1 at 35:25-36:1. Ex. 2 at [0096]; Ex. 1 at 35:25-36:1. Ex. 2 at [0116]-[0117]; Ex. 1 at 35:25-36:1. Ex. 2 at [0118]; Ex. 1 at 36:8-10.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 39	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-47. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]-[0078]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16. <p><u>Schubert (1993)</u></p> <ul style="list-style-type: none"> • Ex. 31 at Abstract. <p><u>Sadtler (1998)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

<p>Claim 39</p>	<p>Prior Art</p> <ul style="list-style-type: none"> Ex. 34 at Abstract. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> Ex. 41 at Abstract. Ex. 41 at 3:40-43. Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23.
<p>Claim 43</p> <p>The method of claim 37, wherein the reaction of the plug-fluids forms a soluble reaction product within at least one plug.</p>	<p>Prior Art</p> <p><i>See claim 1, above.</i></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0316]-[0317]; Ex. 1 at 84:28-85:18. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> Ex. 28 at 2:12-17. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 7:58-68. Id. at Fig. 1. Id. at Fig. 2. Id. at 3:21-37.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 43	<div> <div>Prior Art</div> <ul style="list-style-type: none"> Id. at 3:65-4:22. Ex. 27 at 4552-53. Ex. 24 at Abstract. Id. at 289. Id. at 293. Ex. 26 at 652. Id. at 655. Ex. 38 at [0139] – [0140]. <div> <div>Ghadessy (2001)</div> <div>Katsura (2001)</div> <div>Tawfik (1998)</div> <div>Seki (US 2002/0195463)</div> </div> </div>
Claim 53	<div> <div>Prior Art</div> <div>See claim 37, above.</div> <div>Quake (US 2002/0058332; US 60/233,037)</div> <ul style="list-style-type: none"> Ex. 2 at [0067]; Ex. 1 at 19:17-22. Ex. 2 at [0084]; Ex. 1 at 25:7-11. Ex. 2 at [0214]; Ex. 1 at 62:20-21. </div>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 56	Prior Art
<p>The method of claim 37, wherein the volume of at least one plug is about 1 femtoliter to about 250 nL.</p>	<p>See claim 37, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0003]; Ex. 1 at 2:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 56	Prior Art
	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none">• Ex. 38 at [0025].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
A method of conducting a reaction within at least one plug comprising the steps of:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0012]; Ex. 1 at 5:15-23. • Ex. 2 at [0020]; Ex. 1 at 8:18-20.
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0084]-[0085].
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 7:58-68. • Id. at 3:20-37.
	<p><u>Chow (WO 01/02850 A1)</u></p> <ul style="list-style-type: none"> • Ex. 23 at 33:6-34:2. • Id. at Fig. 1C. • Id. at Fig. 2. • Id. at Fig. 3.
	<p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none"> • Ex. 25 at 5:11-15.
	<p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-17.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Introduction. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
<p>introducing a carrier-fluid into a first microchannel of a device;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:17-25. • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0084]-[0085]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 7:58-68. • Id. at 3:20-37. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-26. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
<p>introducing a stream of a first plug-fluid into a first inlet in fluid communication with the first microchannel and simultaneously introducing a stream of a second plug-fluid into a second inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid at a junction area of the first and second inlets and the first microchannel; wherein: a first plug-fluid comprises a first reagent; a second plug-fluid comprises a second reagent; each plug-fluid is immiscible with the carrier-fluid; each plug comprises both the first and second plug-fluids so that the reaction of the reagents substantially occurs in the plug;</p>	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018]. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0018]; Ex. 1 at 7:20-8:5. • Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. • Ex. 2 at [0318]; Ex. 1 at 85:20-25. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at Fig. 22; Ex. 1 at Fig. 18. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p><u>Kenis (1999)</u></p> <ul style="list-style-type: none"> • Ex. 37 at 83. • Id. at Fig. 2. • Id. at 83. <p><u>Johnson (2000)</u></p> <ul style="list-style-type: none"> • Ex. 45 at Abstract. • Id. at 46-48. • Id. at 51.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<p><u>Erbacher (1999)</u></p> <ul style="list-style-type: none">• Ex. 46 at Abstract.• Id. at 19-20.• Id. at 22-24. <p><u>Whitesides (2000)</u></p> <ul style="list-style-type: none">• Ex. 54 at 942.• Id. at 944-946.• Id. at Fig. 1. <p><u>Weigl (1999)</u></p> <ul style="list-style-type: none">• Ex. 32 at 346.• Id. at Fig. 1. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at Fig. 9D.• Id. at [0084]-[0085].• Id. at [0103].• Id. at [0105].• Id. at [0138]-[0139].• Id. at [0142]-[0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at 7:58-68.• Id. at Fig. 1.

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 2. • Id. at 3:20-37. • Id. at 3:65-4:22. <p><u>Chow (WO 01/02850)</u></p> <ul style="list-style-type: none"> • Ex. 23 at 33:6-34:2. • Id. at Fig. 1C. • Id. at Fig. 2. • Id. at Fig. 3. <p><u>Kopf-Sill (US 5,842,787)</u></p> <ul style="list-style-type: none"> • Ex. 25 at 5:15-19. • Id. at 12:60-13:2. • Id. at 14:3-19. • Id. at Fig. 5B. <p><u>Wang (WO 00/23181)</u></p> <ul style="list-style-type: none"> • Ex. 28 at 2:12-26. • Id. at 3:26-29. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018]. <p><u>Floyd (2002)</u></p> <ul style="list-style-type: none"> Ex. 40 at 92-93.
and each plug is substantially surrounded by carrier.	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 9D. • Id. at [0105]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 6:60-63. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. • Id. at 24. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Seki (US 2002/0195463)</u></p>

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 57	Prior Art
	<ul style="list-style-type: none">• Ex. 38 at [0025].

APPENDIX E – U.S. Patent No. 7,129,091 (Obviousness)

Claim 58	Prior Art
<p>The method of claim 57, wherein each plug initially has a cross section that is substantially the same size as the cross section of the channel at the junction area</p>	<p>See claim 57, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0025].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
<p>A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 11-12. • Ex. 2 at [0020]; Ex. 1 at 8:16-20. • Ex. 2 at [0022]; Ex. 1 at 9:8-16. • Ex. 2 at [0058]; Ex. 1 at 15:18-19. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0119]; Ex. 1 at 37:3-6. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 4. • Ex. 16 at Fig. 1. • Ex. 39 at 1:7-9. • Ex. 39 at 1:20-22. • Ex. 39 at 3:82-86. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 20. • Id. at 36.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0012].• Id. at [0013].• Id. at [0014]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0127].• Id. at [0053].• Id. at [0129]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at Abstract.• Id. at Field of the Invention.• Id. at 3:52-55.• Id. at 4:24-52.• Id. at 3:65-4:2.• Id. at 5:22-25.• Id. at 5:32-40.• Id. at 8:38-51.• Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 35.• Id. at Fig. 6-1.• Id. at 33-34.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 8 at 89. <p><u>Man (2001)</u></p> <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Introduction. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
<p>providing the microfluidic system comprising at least two channels having at least one junction;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-19. • Ex. 2 at [0015]; Ex. 1 at 6:17-19. • Ex. 2 at [0065]; Ex. 1 at 19:7-12. • Ex. 2 at [0068]; Ex. 1 at 19:23-27. • Ex. 2 at [0070]; Ex. 1 at 20:15-18. • Ex. 2 at [0076]; Ex. 1 at 22:21-24. • Ex. 2 at [0084]; Ex. 1 at 25:7-14. • Ex. 2 at [0125]; Ex. 1 at 38:13-16. • Ex. 2 at [0292]; Ex. 1 at 83:8-16. • Ex. 2 at [0323]; Ex. 1 at 86:14-16. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-101.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 16 at 4. Ex. 16 at Fig. 1. Ex. 39 at Figs. 1-5 and 9. Ex. 39 at 1:127-2:3. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 18. Id at 19. Id at 25. Id. at 53. Id. at 56. Id. at 57. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Fabrication. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-57. Id. at Fig. 1.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0139]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Fig. 1. Id. at 3:39-44. Id. at 4:24-30. Id. at 5:58-64. Id. at 7:58-62. Id. at 7:65-68 Id. at 8:38-51. Id. at 8:54-55. Id. at 10:14-19.
<p>flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 1:12-15. Id. at 10:20-28. Id. at 1:21-27.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0012]; Ex. 1 at 5:17-23. • Ex. 2 at [0014]; Ex. 1 at 6:3-5. • Ex. 2 at [0015]; Ex. 1 at 6:19-29. • Ex. 2 at [0020]; Ex. 1 at 8:14-18. • Ex. 2 at [0020]; Ex. 1 at 8:17-21. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0116]; Ex. 1 at 35:19-23. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0287]; Ex. 1 at 82:2-6. • Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. • Ex. 2 at [0323]; Ex. 1 at 86:14-20. • Ex. 2 at [0041]. • Ex. 2 at [0296]. • Ex. 2 at [0301]. • Ex. 2 at Fig. 22. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. • Ex. 39 at 3:4-6. • Ex. 39 at 3:82-86.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 39 at 4:30-33. • Ex. 39 at Fig. 1. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 25. • Id. at 62. • Id. at 107. • Id. at 133. • Id. at 135. • Id. at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0049]. • Id. at [0052]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0129]. • Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. • Id. at 5:32-40. • Id. at 5:58-6:1.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1. • Id. at 88. • Id. at Fig. 6.18. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018].
<p>flowing an oil through the second channel of the at least two channels;</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 10:29-34. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0003]; Ex. 1 at 14-17. Ex. 2 at [0015]; Ex. 1 at 6:21-23. Ex. 2 at [0020]; Ex. 1 at 8:14-16. Ex. 2 at [0022]; Ex. 1 at 9:14-16. Ex. 2 at [0064]; Ex. 1 at 18:22-25. Ex. 2 at [0113]; Ex. 1 at 34:26-29. Ex. 2 at [0125]; Ex. 1 at 38:16-19. Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. Ex. 2 at [0287]; Ex. 1 at 82:2-3. Ex. 2 at [0323]; Ex. 1 at 86:14-20. Ex. 2 at [0301].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 18. Id. at 66-7. Id. at 89. Id. at 133. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0047]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at [0084]. Id. at [0085]. Id. at [0105]. Id. at [0138]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 9:35-40. Id. at Abstract. Id. at 3:24-25.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 3:39-44. • Id. at 4:3-5. • Id. at 5:38-40. • id. at 6:60-62. • Id. at 7:36-39. • Id. at 9:35-40. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:63-67. • Id. at 6:36-50. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4136. • Id. at 4164. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
<p>forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels,</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:23-28. • Id. at 10:31-35. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at [0012]; Ex. 1 at 5:15-23. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0020]; Ex. 1 at 8:18-9:2. • Ex. 2 at [0022]; Ex. 1 at 9:8-16. • Ex. 2 at [0058]; Ex. 1 at 15:18-19. • Ex. 2 at [0070]; Ex. 1 at 20:15-21. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [0125]; Ex. 1 at 38:13-16. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. • Ex. 2 at [0015]; Ex. 1 at 7:7-10. • Ex. 2 at Figs. 16A, 16B, 17A, and 17B; Ex. 1 at Fig. 16A, 16B, 17A,

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p>and 17B.</p> <ul style="list-style-type: none"> • Ex. 2 at [0041]. • Ex. 2 at [0042]. • Ex. 2 at [0296]. • Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12 • Ex. 16 at Abstract. • Ex. 39 at Figs. 1-5 and 9. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 19. • Id. at 62. • Id. at 133. • Id. at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0049]. • Id. at [0052]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0105].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Id. at [0129].• Id. at [0144].• Ex. 6 at 4:24-52.• Id. at 6:60-63.• Id. at Figs. 1 and 2.• Id. at 3:24-25.• Id. at 3:39-44.• Id. at 4:3-5.• Id. at 4:24-35.• Id. at 5:38-40.• Id. at 5:58-64.• Id. at 6:60-62.• Id. at 6:65-67.• Id. at 7:20-21.• Id. at 7:58-62.• Id. at 9:35-38.• Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 33-35.• Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none">• Ex. 8 at 90.• Id. at Table 8.1.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p data-bbox="280 678 313 856"><u>Burns (1996)</u></p> <ul data-bbox="350 982 423 1262" style="list-style-type: none"> • Ex. 36 at 5556-59 • Id. at Fig. 1. <p data-bbox="459 600 492 934"><u>Nisisako Abstract (2001)</u></p> <ul data-bbox="529 835 678 1262" style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p data-bbox="716 663 748 871"><u>Thorsen (2001)</u></p> <ul data-bbox="786 1020 818 1262" style="list-style-type: none"> • Ex. 13 at 4136. <p data-bbox="854 663 886 871"><u>Nisisako (2002)</u></p> <ul data-bbox="924 993 997 1262" style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p data-bbox="1034 678 1066 856"><u>Burns (2001)</u></p> <ul data-bbox="1104 1010 1177 1262" style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p data-bbox="1213 655 1245 879"><u>Ghadessy (2001)</u></p> <ul data-bbox="1282 978 1315 1262" style="list-style-type: none"> • Ex. 27 at 4552-53.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Katsura (2001)</u></p> <ul style="list-style-type: none">• Ex. 24 at Abstract.• Id. at 289.• Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none">• Ex. 26 at 652.• Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none">• Ex. 43 at 2018.• Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none">• Ex. 38 at [0018].
the plug being substantially surrounded by an oil flowing through the channel,	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0100]; Ex.1 at 30:2-3.• Ex. 2 at [0191]-[0192]; Ex. 1 at 27:22-28:6.• Ex. 2 at [0041].• Ex. 2 at [0042].• Ex. 2 at [0093].• Ex. 2 at [0296].• Ex. 2 at Figs. 18, 19J, and 19L. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 16 at 12.• Ex. 16 at Abstract.• Ex. 39 at Figs. 1 and 3-6.• Ex. 39 at 3:102-104. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 19.• Id. at 149. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at Fig. 9D.• Id. at [0105]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex.17 at Abstract.• Id. at Experimental.• Id. at Fig. 1.• Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract.• Id. at Fig. 1.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Fig. 2. Id. at 6:60-62. Id. at 6:65-67. Id. at 7:20-21.
<p>wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule; and</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 10:23-28. Id. at 1:21-27. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0004]; Ex. 1 at 2:10-14. Ex. 2 at [0020]; Ex. 1 at 8:14-21. Ex. 2 at [0021]; Ex. 1 at 9:3-6. Ex. 2 at [0050]; Ex. 1 at 13:18-19. Ex. 2 at [0052]; Ex. 1 at 13:23-29. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0108]; Ex. 1 at 33:12-14. Ex. 2 at [0113]; Ex. 1 at 34:23-26. Ex. 2 at [0120]; Ex. 1 at 37:12-19.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.• Ex. 16 at 12.• Ex. 16 at Abstract.• Ex. 39 at 3:82-86. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 2.• Id. at 17.• Id. at 18.• Id. at 19.• Id. at 62.• Id. at 133.• Id. at 147-8. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0049].• Id. at [0052]. <p style="text-align: center;"><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0105].• Id. at [0129].• Id. at [0144].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at Abstract. • Id. at 3:52-55. • Id. at 3:65-4:2. • Id. at 4:24-52. • Id. at 5:22-30. • Id. at 6:60-63. • Id. at 9:11-17. • Id. at 9:57-61. • Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59 • Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
providing conditions suitable for the autocatalytic reaction in the at least one plug such that at least one substrate molecule is amplified.	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. • Id. at 9:55-10:2. • Id. at 10:15-19. • Id. at 10:53-56. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0080]; Ex. 1 at 24:12-13.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 39 at 2:44-50.• Id. at 3:57-60.• Id. at 3:70-73. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0012].• Id. at [0013].• Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at Abstract.• Id. at Field of the invention.• Id. at 3:20-40.• Id. at 4:37-43.• Id. at 4:62-65.• Id. at 5:42-44.• Id. at 6:32-40.• Id. at 6:44-54.• Id. at Fig. 1.• Id. at 7:55-68.• Id. at 8:1-11.• Id. at 9:19-34.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p data-bbox="280 674 313 856"><u>Curcio (2002)</u></p> <ul data-bbox="350 1020 423 1262" style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p data-bbox="459 688 492 842"><u>Man (2001)</u></p> <ul data-bbox="529 1041 638 1262" style="list-style-type: none"> • Ex. 8 at 93. • Id. at 97-100. • Id. at 104. <p data-bbox="675 678 708 852"><u>Burns (1996)</u></p> <ul data-bbox="745 978 818 1262" style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p data-bbox="854 653 886 877"><u>Ghadessy (2001)</u></p> <ul data-bbox="924 978 956 1262" style="list-style-type: none"> • Ex. 27 at 4552-53. <p data-bbox="993 663 1026 867"><u>Katsura (2001)</u></p> <ul data-bbox="1063 978 1172 1262" style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p data-bbox="1209 669 1242 861"><u>Tawfik (1998)</u></p> <ul data-bbox="1279 1041 1352 1262" style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 1	Prior Art
	<p><u>Chiou (2001)</u></p> <ul style="list-style-type: none">• Ex. 43 at 2018.• Ex. 43 at 2019.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 2	Prior Art
<p>The method of claim 1, wherein the at least one substrate molecule is a single biological molecule.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0012]; Ex. 1 at 5:17-19. • Ex. 2 at [0015]; Ex. 1. at 6:29-7:5. • Ex. 2 at [0022]; Ex. 1 at 9:20-23. • Ex. 2 at [0020]; Ex. 1 at 8:18-21. • Ex. 2 at [0120]; Ex. 1 at 37:12-19. • Ex. 2 at [0182]; Ex. 1 at 55:9-12. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 36. • Id. at 123. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0012]. • Id. at [0013]. • Id. at [0014]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0129]. • Id. at [0144].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 2	Prior Art
	<p><u>Lagally (2001)</u></p> <ul style="list-style-type: none"> Ex. 18 at Abstract.
Claim 3	Prior Art
<p>The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.</p>	<p><i>See claim 2, above.</i></p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 1:12-15. Id. at 10:20-25. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0050]; Ex. 1 at 13:18-19. Ex. 2 at [0052]; Ex. 1 at 13:23-29. Ex. 2 at [0058]; Ex. 1 at 15:18-19. Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 3:70-86. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 135. Id. at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 3	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Id. at [0014]. • Ex. 6 at Abstract. • Id. at Field of the invention. • Id. at 3:52-55. • Id. at 3:65-4:2. • Id. at 4:24-52. • Id. at 5:22-25. • Id. at 9:11-17. • Id. at 9:57-61, • Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 89-90. • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 3	Prior Art
	<div> <ul style="list-style-type: none"> Ex. 27 at 4552-53. </div> <div> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. </div> <div> <ul style="list-style-type: none"> Ex. 26 at 652. Id. at 655. </div> <div> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. </div> <div> <p><u>Ghadessy (2001)</u></p> <p><u>Katsura (2001)</u></p> <p><u>Tawfik (1998)</u></p> <p><u>Chiou (2001)</u></p> </div>
Claim 4	Prior Art
The method of claim 1, wherein the providing step includes heating.	<div> <p>See claim 1, above.</p> </div> <div> <ul style="list-style-type: none"> Ex. 9 at 1:61-64. Id. at 9:61-10:6. Id. at 10:15-19. Id. at 10:48-51. </div> <div> <p><u>Haff (US 6,033,880)</u></p> </div>

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 4	Prior Art
	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 5; Ex 39 at 2:44-52. Ex. 39 at 3:57-60. Ex. 39 at 3:70-73. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Abstract. Id. at Field of the invention. Id. at 3:26-32. Id. at 4:24-52. Id. at 4:62-65. Id. at 5:42-44. Id. at 6:32-40. Id. at 6:44-51. Id. at 8:1-5. Id. at 9:19-25.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 4	Prior Art
	<p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 97-100. • Id. at 104. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59 • Id. at Fig. 1. <p><u>Shaw Stewart (WO 84/02000)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 5. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 5	Prior Art
<p>The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.</p>	<p><i>See claim 1, above.</i></p> <ul style="list-style-type: none"> • Ex. 9 at 8:18-27. • Id. at 25:34-40. • Id. at 25:41-44. • Id. Fig. 30. • Id. at 26:1-6. • Id. at 26:23-26. <p><u>Haff (US 6,033,880)</u></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0016]; Ex. 1 at 7:11-14. • Ex. 2 at [0018]; Ex. 1 at 8:3-5. • Ex. 2 at [0020]; Ex. 1 at 8:23-27. • Ex. 2 at [0022]; Ex. 1 at 9:8-12. • Ex. 2 at [0025]; Ex. 1 at 10:4-7. • Ex. 2 at [0054]; Ex. 1 at 14:20-28. • Ex. 2 at [0069]; Ex. 1 at 20:3-5. • Ex. 2 at [0076]; Ex. 1 at 22:21-26. • Ex. 2 at [0078]; Ex. 1 at 23:12-20. • Ex. 2 at [0086]; Ex. 1 at 25:28-26:1. • Ex. 2 at [0106]; Ex. 1 at 32:6-7. • Ex. 2 at [0110]; Ex. 1 at 34:6-12. • Ex. 2 at [0237]; Ex. 1 at 69:10-11. • Ex. 2 at Figs. 2A, 2B, 14, and 15; Ex. 1 at Figs. 2A, 2B, 14, and 15.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 5	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 6-7; Ex. 39 at 2:94-105. Ex. 39 at 2:59-66. Ex. 39 at 2:69-70. Ex. 39 at 2:90-92. Ex. 39 at 3:57-60. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 20. Id. at 28. Id. at 61. Id. at 62. Id. at 64. Id. at 66. Id. at 68. Id. at 70. Id. at 71. Id. at 73. Id. at 142. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 11. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 100-01. Id. at Fig. 8.10.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 5	Prior Art
	<p data-bbox="280 678 313 856"><u>Burns (1996)</u></p> <ul data-bbox="350 976 383 1260" style="list-style-type: none"> • Ex. 36 at 5558-60. <p data-bbox="402 678 435 856"><u>Chiou (2001)</u></p> <ul data-bbox="456 1016 488 1260" style="list-style-type: none"> • Ex. 43 at 2019. <p data-bbox="508 611 540 930"><u>Seki (US 2002/0195463)</u></p> <ul data-bbox="561 997 594 1260" style="list-style-type: none"> • Ex. 38 at [0143]. <p data-bbox="631 611 664 919"><u>Corbett (US 5,270,183)</u></p> <ul data-bbox="701 997 889 1260" style="list-style-type: none"> • Ex. 6 at 4:53-55. • Id. at 6:18-20. • Id. at 8:13-24. • Id. at 8:58-62. • Id. at 10:12-14.
Claim 6	Prior Art
<p data-bbox="1075 1377 1140 1904">The method of claim 1, wherein the oil is fluorinated oil.</p>	<p data-bbox="1075 1075 1107 1320">See claim 1, above.</p> <p data-bbox="1144 485 1177 1045"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul data-bbox="1214 747 1403 1260" style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0094]; Ex. 1 at 28:21-23. • Ex. 2 at [0096]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0118]; Ex. 1 at 36:8-10.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 6	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:41. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 18. • Id. at 89. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:63-66. • Id. at 6:36-50. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0047]. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 36. <p><u>Shaw Stewart (WO 84/02000)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 6	Prior Art
	<ul style="list-style-type: none">• Ex. 41 at 3:47-50.• Ex. 41 at 7:57-8:6.• Ex. 41 at 9:24-51.• Ex. 41 at 11:5-23. <p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none">• Ex. 42 at Abstract.• Ex. 42 at 2:29-34.• Ex. 42 at 3:16-23.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 7	Prior Art
<p>The method of claim 1, wherein the carrier fluid further comprises a surfactant.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0094]; Ex. 1 at 28:21-23. • Ex. 2 at [0096]; Ex. 1 at 35:2-36:1. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. • Ex. 2 at [0020]. • Ex. 2 at [0022]. • Ex. 2 at [0041]. • Ex. 2 at [0042]. • Ex. 2 at [0095]. • Ex. 2 at [0300]. • Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-48. • Ex. 39 at 2:19-26. • Ex. 39 at 4:26-29. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 7	Prior Art
	<p><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23.

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 8	Prior Art
<p>The method of claim 7, wherein the surfactant is fluorinated surfactant.</p>	<p><i>See claim 7, above.</i></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 39 at 1:39-41. • Ex. 29 at Abstract. • Id. at 1:13-16. • Ex. 31 at Abstract. • Ex. 34 at Abstract. • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <p><u>Delpuech (US 5,185,099)</u></p> <p><u>Schubert (1993)</u></p> <p><u>Sadtler (1998)</u></p> <p><u>Krafft (U.S. 5,980,936)</u></p>
Claim 11	Prior Art

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 11	Prior Art
<p>The method of claim 1, wherein the at least one plug is substantially spherical in shape.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0091]; Ex. 1 at 27:22-27. • Ex. 2 at [0092]; Ex. 1 at 28:19-20. • Ex. 2 at [0292]; Ex. 1 at 83:13-16. • Ex. 2 at [0315]; Ex. 1 at 84:16-24. • Ex. 2 at Figs. 14, 15, 16B, 18, 19, and 22; Ex. 1 at Figs. 14, 15, and 16B. • Ex. 2 at [0093]. • Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 4. • Ex. 16 at Fig. 1. • Ex. 39 at Figs. 1 and 3-6. • Ex. 39 at 3:102-104. • Ex. 39 at 3:128-4:4. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 19. • Id. at 149. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0105].

APPENDIX F – U.S. Patent No. 8,304,193 (Obviousness)

Claim 11	Prior Art
	<p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
A method for conducting a reaction in plugs in a microfluidic system, comprising the steps of:	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at Abstract; Ex. 1 at Abstract. Ex. 2 at [0020]; Ex. 1 at 8:16-20. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0119]; Ex. 1 at 37:5-6. Ex. 2 at [0095]. Ex. 2 at [0296]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 4. Ex. 16 at Fig. 1. Ex. 39 at 3:82-86. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 17. Id. at 20. Id. at 36. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0012].

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Id. at [0013].• Id. at [0014]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0127].• Id. at [0053].• Id. at [0129]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at Abstract.• Id. at 5:58-6:1.• Id. at 6:60-62.• Id. at 7:20-21.• Id. at 7:58-62.• Id. at 8:38-46.• Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 33-35.• Id. at Fig. 6-I. <p><u>Man (2001)</u></p> <ul style="list-style-type: none">• Ex. 8 at 89. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Introduction.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
<p>providing the microfluidic system comprising at least two channels having at least one junction;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-19. • Ex. 2 at [0015]; Ex. 1 at 6:17-19. • Ex. 2 at [0068]; Ex. 1 at 19:23-27. • Ex. 2 at [0070]; Ex. 1 at 20:15-18. • Ex. 2 at [0084]; Ex. 1 at 25:7-14.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 2 at [0125]; Ex. 1 at 25:13-16. • Ex. 2 at [0292]; Ex. 1 at 83:8-16. • Ex. 2 at [0323]; Ex. 1 at 86: 14-16. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-101. • Ex. 16 at 4. • Ex. 16 at Fig. 1; Ex. 39 at Figs. 1-5 and 9. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 18. • Id. at 19. • Id. at 25. • Id. at 53. • Id. at 56. • Id. at 57. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Fabrication. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4136. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 1. <p style="text-align: center;"><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-57. • Id. at Fig. 1. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018]. <p style="text-align: center;"><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at Fig. 1. • Id. at 5:58-6:1. • Id. at 8:38-46.
continuously flowing an aqueous fluid containing at least one biological molecule and at least one reagent for conducting the reaction between the biological molecule and the at least one reagent through a first channel of the at	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:20-28. • Id. at 1:21-27.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
least two channels;	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0012]; Ex. 1 at 5:17-23. • Ex. 2 at [0014]; Ex. 1 at 6:3-5. • Ex. 2 at [0020]; Ex. 1 at 8:14-18. • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0113]; Ex. 1 at 34:23-35:2. • Ex. 2 at [0116]; Ex. 1 at 35:19-23. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0166]; Ex. 1 at 50:7-13. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. • Ex. 39 at 3:4-6. • Ex. 39 at 4:30-33. • Ex. 39 at Fig. 1. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 20. • Id. at 62. • Id. at 133. • Id. at 135. • Id. at 147. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0049]. • Id. at [0052]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0129]. • Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. • Id. at 5:32-40. • Id. at 5:58-6:1. • Id. at 6:60-62. • Id. at 6:65-67. • Id. at 7:20-21. • Id. at 7:58-62. • Id. at 8:38-46. • Id. at 8:54-55. • Id. at 9:35-38. • Id. at 10:14-19.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<div> <div> <ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1. </div> <div> <p><u>Curcio (2002)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 8 at 90. Id. at Table 8.1. Id. at 88. Id. at Fig. 6.18. </div> <div> <p><u>Man (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig. 1. </div> <div> <p><u>Burns (1996)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 17 at Experimental. </div> <div> <p><u>Nisisako Abstract (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 13 at 4136. </div> <div> <p><u>Thorsen (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at 25. </div> <div> <p><u>Nisisako (2002)</u></p> </div> </div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
continuously flowing a carrier fluid immiscible with the aqueous fluid through the second channel of the at least two channels;	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:29-34. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0020]; Ex. 1 at 8:14-18. • Ex. 2 at [0022]; Ex. 1 at 9:14-16. • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0070]; Ex. 1 at 20:15-21. • Ex. 2 at [0113]; Ex. 1 at 34:26-29. • Ex. 2 at [0116]; Ex. 1 at 35:19-27. • Ex. 2 at [0125]; Ex. 1. at 38:16-19. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at [0287]; Ex. 1 at 82:2-3.• Ex. 2 at [0290]; Ex. 1 at 82:22-27.• Ex. 2 at [0301].• Ex. 2 at [0323]. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.• Ex. 16 at 4-5.• Ex. 16 at 12.• Ex. 16 at Abstract.• Ex. 39 at Fig. 1.• Ex. 39 at 3:82-86.• Ex. 39 at 3:102-104. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 2.• Id. at 17.• Id. at 18.• Id. at 61. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0047]. <p style="text-align: center;"><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at [0084].• Id. at [0085].

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at [0105]. • Id. at [0138]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at Abstract. • Id. at 3:20-44. • Id. at 4:3-5. • Id. at 4:24-35. • Id. at 5:38-40. • Id. at 6:60-62. • Id. at 8:46-51. • Id. at 9:35-40. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:63-67. • Id. at 6:36-50. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at 25.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
<p>forming at least one plug of the aqueous fluid containing the at least one biological molecule and the at least one reagent by partitioning the aqueous fluid with the flowing immiscible carrier fluid at the junction of the at least two channels,</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:23-28. • Id. at 10:31-35. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:17-2:2. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0015]; Ex. 1 at 7:7-10. • Ex. 2 at [0020]; Ex. 1 at 8:18-27. • Ex. 2 at [0070]; Ex. 1 at 20:15-21. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [0125]; Ex. 1 at 38:13-16. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0287]; Ex. 1 at 82:2-3.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12 Ex. 16 at Abstract. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 17. Id. at 18. Id. at 19. Id. ta 62. Id. at 133. Id. at 147. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0049]. Id. at [0052]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0105]. Id. at [0129]. Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 6 at 3:24-25.• Id. at 4:3-5.• Id. at 4:24-52.• Id. at 5:38-40.• Id. at 5:58-6:1.• Id. at 6:60-63.• Id. at 6:65-67.• Id. at 7:20-21.• Id. at 7:58-62.• Id. at 8:54-55.• Id. at 9:35-38.• Id. at Fig. 2. <p style="text-align: center;"><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 33-35.• Id. at Fig. 6-1. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Abstract.• Id. at Experimental.• Id. at Fig. 1.• Id. at Results and Discussion. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4136. <p style="text-align: center;"><u>Nisisako (2002)</u></p>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 90. Id. at Table 8.1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> Ex. 26 at 652.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 655. <p style="text-align: center;"><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018]. <p style="text-align: center;"><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> • Ex. 47 at 7:64-8:13. • Id. at 8:28-34.
<p>the plug being substantially surrounded by the immiscible carrier fluid flowing through the channel,</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0100]; Ex. 1 at 30:2-3. • Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [00241]; Ex. 1 at 70:22-25. • Ex. 2 at [0041]. • Ex. 2 at [0042]. • Ex. 2 at Figs. 19J and 19L. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. • Ex. 39 at Fig. 1. • Ex. 39 at 3:102-104.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 19.• Id. at 62.• Id. at 149. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0053].• Id. at Fig. 9D.• Id. at [0105]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Abstract.• Id. at Experimental.• Id. at Fig. 1.• Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4136. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract.• Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 10-11.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 14. • Ex. 38 at [0018]. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p>
<p>wherein the at least one plug comprises at least one biological molecule and the at least one reagent for conducting the reaction with the at least one biological molecule; and</p>	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:20-28 • Id. at 1:21-27. <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0014]; Ex. 1 at 6:10-14. • Ex. 2 at [0020]; Ex. 1 at 8:14-21. • Ex. 2 at [0058]; Ex. 1 at 15:18-19. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0106]; Ex. 1 at 32:14-16. • Ex. 2 at [0113]; Ex. 1 at 34:23-35:2. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0323]. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:70-86. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 133. • Id. at 147-8.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0049]. Id. at [0052]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0105]. Id. at [0129]. Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 3:65-4:5. Id. at 4:24-52. Id. at 5:32-37. Id. at 5:38-40. Id. at 5:58-6:1. Id. at 6:60-63. Id. at 8:38-46. Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 90. Id. at Table 8.1.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<div> <div> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig.1. </div> <div> <p><u>Burns (1996)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 27 at 4552-53. </div> <div> <p><u>Ghadessy (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. </div> <div> <p><u>Katsura (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 26 at 652. Id. at 655. </div> <div> <p><u>Tawfik (1998)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. </div> <div> <p><u>Chiou (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 38 at [0145]. </div> <div> <p><u>Seki (US 2002/0195463)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 47 at 7:64-8:13. </div> <div> <p><u>Vogelstein (US 6,440,706)</u></p> </div> </div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
<p>providing conditions suitable for the reaction in the at least one plug involving the at least one biological molecule and the at least one reagent to form a reaction product.</p>	<ul style="list-style-type: none"> • Id. at 8:28-34. <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:23-28. • Id. at 1:21-27. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0012]; Ex. 1 at 5:17-23. • Ex. 2 at [0020]; Ex. 1 at 8:14-21. • Ex. 2 at [0058]; Ex. 1 at 15:18-19. • Ex. 2 at [0078]; Ex. 1 at 23:12-20. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0106]; Ex. 1 at 32:14-16. • Ex. 2 at [0113]; Ex. 1 at 34:23-35:2. • Ex. 2 at [0120]; Ex. 1 at 37:12-19. • Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. • Ex. 2 at [0296]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 2:44-50. • Ex. 39 at 3:57-60. • Ex. 39 at 3:70-73. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 147-8.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0012]. • Id. at [0013]. • Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at Abstract. • Id. at Field of the invention. • Id. at 3:20-44. • Id. at 4:6-18. • Id. at 4:37-43. • Id. at 5:8-11. • Id. at 5:42-44. • Id. at 7:55-68. • Id. at 8:1-5. • Id. at 8:46-51. • Id. at 9:19-20. • Id. at Fig.1. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-I. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 93. • Id. at 97-100.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 104. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 1	Prior Art
	<p><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> • Ex. 47 at 8:21-24. • Id. at 8:40-44.
Claim 2	Prior Art
<p>The method according to claim 1, wherein the at least one biological molecule is DNA or RNA.</p>	<p>See claim 1, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:23-25. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0020]; Ex. 1 at 8:18-21. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0050]; Ex. 1 at 13:18-19. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. • Ex. 2 at [0058]; Ex. 1 at 15:18-16:3. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0108]; Ex. 1 at 33:12-14. • Ex. 2 at [0109]; Ex. 1 at 33:28-34:2. • Ex. 2 at [0113]; Ex. 1 at 34:23-26. • Ex. 2 at [0166]; Ex. 1 at 50:7-13. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:70-86. • Ex. 39 at 3:82-86.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 2	Prior Art
	<div> <ul style="list-style-type: none"> Ex. 39 at 4:30-33. </div> <div> <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 135. Id. at 147-8. </div> <div> <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013]. Id. at [0014]. </div> <div> <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Abstract. Id. at Field of the invention. Id. at 3:52-55. Id. at 3:65-4:2. Id. at 4:24-52. Id. at 5:22-25. Id. at 9:11-17. Id. at 9:57-61. Id. at 10:14-19. </div> <div> <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1. </div> <div> <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 89-90. </div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 2	Prior Art
	<ul style="list-style-type: none"> • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 3	Prior Art
<p>The method according to claim 2, wherein the reaction is an autocatalytic reaction.</p>	<p>See claim 2, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0080]; Ex. 1 at 1 24:12-13. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013]. Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Abstract. Id. at Field of the invention. Id. at 3:52-55. Id. at 3:65-4:2. Id. at 4:24-52. Id. at 5:22-25. Id. at 9:11-17. Id. at 9:57-61. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 3	Prior Art
	<div> <div> <ul style="list-style-type: none"> Ex. 8 at 89-90. Id. at Table 8.1. </div> <div> <p><u>Man (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig. 1. </div> <div> <p><u>Burns (1996)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 27 at 4552-53. </div> <div> <p><u>Ghadessy (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. </div> <div> <p><u>Katsura (2001)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 26 at 652. Id. at 655. </div> <div> <p><u>Tawfik (1998)</u></p> </div> </div> <div> <div> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. </div> <div> <p><u>Chiou (2001)</u></p> </div> </div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 4	Prior Art
<p>The method according to claim 2, wherein the reaction is a polymerase chain reaction.</p>	<p>See claim 2, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. • Id. at 10:20-25. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0013]. • Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at Abstract. • Id. at Field of the invention. • Id. at 3:52-55. • Id. at 3:65-4:2. • Id. at 4:24-52. • Id. at 5:22-25. • Id. at 9:11-17. • Id. at 9:57-61. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 4	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 89-90. • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 5	Prior Art
<p>The method according to claim 1, wherein the reaction is an enzymatic reaction.</p>	<p>See claim 1, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0021]; Ex. 1 at 9:3-7. Ex. 2 at [0058]; Ex. 1 at 15:29-16:2. Ex. 2 at [0078]; Ex. 1 at 23:16-20. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0099]; Ex. 1 at 29:8-10. Ex. 2 at [0106]; Ex. 1 at 32:14-16. Ex. 2 at [0108]; Ex. 1 at 33:12-14. Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0045]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 3:82-86. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 43. Id. at 89. Id. at 135. Id. at 147-8.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 5	Prior Art
	<p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013]. Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Abstract. Id. at Field of the invention. Id. at 3:52-55. Id. at 3:65-4:2. Id. at 4:24-52. Id. at 5:22-25. Id. at 5:32-37. Id. at 9:11-17. Id. at 9:57-61. Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 89-90. Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig. 1.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 5	<div> <div>Prior Art</div> <div> <div>Chiou (2001)</div> <div> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. </div> </div> <div> <div>Seki (US 2002/0195463)</div> <div> <ul style="list-style-type: none"> Ex. 38 at [0139]. </div> </div> </div>
Claim 8	<div> <div>Prior Art</div> <div> <div>Haff (US 6,033,880)</div> <div> <p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 9 at 10:29-35. </div> </div> <div> <div>Quake (US 2002/0058332; US 60/233,037)</div> <div> <ul style="list-style-type: none"> Ex. 2 at [0003]; Ex. 1 at 1:14-17. Ex. 2 at [0014]; Ex. 1 at 6:14-16. Ex. 2 at [0015]; Ex. 1 at 6:21-23. Ex. 2 at [0020]; Ex. 1 at 8:27-28. Ex. 2 at [0022]; Ex. 1 at 9:18-20. Ex. 2 at [0100]; Ex. 1 at 30:2-3. Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. Ex. 2 at [0287]; Ex. 1 at 82:2-6. Ex. 2 at [0290]; Ex. 1 at 82:22-27. Ex. 2 at [0323]; Ex. 1 at 86:14-17. Ex. 2 at [0096]. Ex. 2 at [0300]. </div> </div> </div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 8	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 4; Ex. 39 at 1:41. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 18. Id. at 45. Id. at 66-7. Id. at 89. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> Ex. 14 at 3:63-67. Id. at 6:36-50. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0047]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Experimental. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4164. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at 25.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 8	Prior Art
	<div><div><div>• Ex. 35 at 10-11.</div></div><div><div><div><u>Burns (2001)</u></div><div><u>Corbett (US 5,270,183)</u></div><div><u>Chiou (2001)</u></div></div><div><div>• Ex. 6 at 9:35-40.</div><div>• Ex. 43 at 2018.</div><div>• Ex. 43 at 2019.</div></div></div></div>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 9	Prior Art
The method according to claim 8, wherein the oil comprises a surfactant.	<p>See claim 8, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0022]; Ex. 1 at 9:18-20.• Ex. 2 at [0117]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0094]; Ex. 1 at 28:21-23.• Ex. 2 at [0118]; Ex. 1 at 36:8-10.• Ex. 2 at [0096]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0020].• Ex. 2 at [0300]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 4; Ex. 39 at 1:44-48.• Ex. 39 at 2:19-26.• Ex. 39 at 4:26-29. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none">• Ex. 19 at [0041].• Id. at [0075].• Id. at [0076].• Id. at [0077]. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none">• Ex. 15 at 4600-01.• Id. at 4604.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 9	Prior Art
	<p><u>Thorsen (2001)</u></p> <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4164.• Ex. 41 at Abstract.• Ex. 41 at 3:40-43.• Ex. 41 at 3:47-50.• Ex. 41 at 7:57-8:6.• Ex. 41 at 9:24-51.• Ex. 41 at 11:5-23.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 10	Prior Art
<p>The method according to claim 9, wherein the surfactant is a fluorosurfactant.</p>	<p>See claim 9, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 1:39-41. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 18. • Id. at 89. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16. <p><u>Schubert</u></p> <ul style="list-style-type: none"> • Ex. 31 at Abstract. <p><u>Sadtler</u></p> <ul style="list-style-type: none"> • Ex. 34 at Abstract. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 10	Prior Art
	<ul style="list-style-type: none"> • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23.
Claim 11	Prior Art
<p>The method according to claim 8, wherein the oil is a fluorinated oil.</p>	<p>See claim 8, above.</p> <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:39-41. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 18. <p style="text-align: center;"><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:63-67. • Id. at 6:36-50. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0047].

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 11	Prior Art
	<ul style="list-style-type: none">• Ex. 33 at 36.• Ex. 13 at 4164.• Ex. 16 at 4.• Ex. 41 at Abstract.• Ex. 41 at 3:40-43.• Ex. 41 at 3:47-50.• Ex. 41 at 7:57-8:6.• Ex. 41 at 9:24-51.• Ex. 41 at 11:5-23. <p><u>Curcio (2002)</u></p> <p><u>Thorsen (2001)</u></p> <p><u>Shaw Stewart (WO 84/02000)</u></p> <p><u>Krafft (U.S. 5,980,936)</u></p>
Claim 13	Prior Art
The method according to claim 1, wherein the providing step includes heating.	<p>See claim 1, above.</p> <ul style="list-style-type: none">• Ex. 9 at 1:61-64.• Id. at 9:61-10:6.• Id. at 10:15-19.• Id. at 10:48-51. <p><u>Haff (US 6,033,880)</u></p>

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 13	Prior Art
	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 5; Ex 39 at 2:44-52. Ex. 39 at 3:57-60. Ex. 39 at 3:70-73. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 147-8. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0013]. Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Abstract. Id. at Field of the invention. Id. at 3:21-23. Id. at 3:26-32. Id. at 3:39-44. Id. at 4:6-18. Id. at 4:24-52. Id. at 5:8-11. Id. at 5:42-44. Id. at 8:1-5. Id. at 8:46-51.

APPENDIX G – U.S. Patent No. 8,329,407 (Obviousness)

Claim 13	Prior Art
	<ul style="list-style-type: none"> • Id. at 9:19-20. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 97-100. • Id. at 104. <p><u>Shaw Stewart (WO 84/02000)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 5. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
<p>A method comprising the steps of: providing a microfluidic system comprising one or more channels;</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 9:56-63. • Id. at Fig. 2. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-19. • Ex. 2 at [0015]; Ex. 1 at 6:17-19. • Ex. 2 at [0022]; Ex. at 9:8-16. • Ex. 2 at [0292]; Ex. 1 at 83:8-16. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 89. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-101. • Ex. 16 at 4. • Ex. 16 at Fig. 1; Ex. 39 at Figs. 1-5 and 9. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 19. • Id. at 25.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 36. • Id. at 53. • Id. at 56. • Id. at 57. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-57. • Id. at 5559. • Id. at Figs. 1, 3A, and 4A-4D. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at Fig. 1. • Id. at 5:58-64. • Id. at 7:58-62. <p style="text-align: center;"><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0004]. <p style="text-align: center;"><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
providing within the one or more channels a continuously flowing carrier fluid comprising an oil and	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:29-34. <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0014]; Ex. 1 at 6:3-5. • Ex. 2 at [0015]; Ex. 1 at 6:21-23. • Ex. 2 at [0020]; Ex. 1 at 8:14-16. • Ex. 2 at [0022]; Ex. 9 at 14-16. • Ex. 2 at [0064]; Ex. 1 at 18:22-25. • Ex. 2 at [0113]; Ex. 1 at 34:26-29. • Ex. 2 at [0114]; Ex. 1 at 35:3-4. • Ex. 2 at [0287]; Ex. 1 at 82:2-6.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 2 at [0323]; Ex. 1 at 86:14-17. • Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> • Ex. 44 at 2. • Id. at 17. • Id. at 18. • Id. at 61. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 3:63-67. • Id. at 6:36-50. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0047]. <p><u>Shaw Stewart (WO 84/02000)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4-5. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Experimental.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163-64. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at 25. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 9:35-40. Id. at Abstract. Id. at 3:24-25. Id. at 3:39-44. Id. at 4:3-5. Id. at 5:38-40. Id. at 5:58-6:1. Id. at 7:58-62. Id. at 8:46-51. Id. at 9:35-38. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018].
<p>a continuously flowing aqueous fluid comprising target DNA or RNA molecules and at least one other molecule in the fluid that can react with the target DNA or RNA molecules under conditions in which the target DNA or RNA molecules and the other molecules in the fluid do not react with each other;</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> Ex. 9 at 10:20-28. Id. at 1:21-27. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at Abstract; Ex. 1 at 2:11-13. Ex. 2 at [0003]; Ex. 1 at 1:14-19. Ex. 2 at [0004]; Ex. 1 at 10-13. Ex. 2 at [0012]; Ex. 1 at 5:17-23. Ex. 2 at [0014]; Ex. 1 at 6:3-5. Ex. 2 at [0015]; Ex. 1 at 6:19-29. Ex. 2 at [0020]; Ex. 1 at 8:14-18. Ex. 2 at [0022]; Ex. 1 at 9:8-18. Ex. 2 at [0050]; Ex. 1 at 18-19. Ex. 2 at [0052]; Ex. 1 at 13:23-29. Ex. 2 at [0058]; Ex. 1 at 15:18-16:3. Ex. 2 at [0064]; Ex. 1 at 18:22-25. Ex. 2 at [0080]; Ex. 1 at 24:12-13. Ex. 2 at [0113]; Ex. 1 at 34:23-26. Ex. 2 at [0115]; Ex. 1 at 35:11-17. Ex. 2 at [0116]; Ex. 1 at 35:19-23. Ex. 2 at [0170]; Ex. 1 at 51:19-52:5. Ex. 2 at [0287]; Ex. 1 at 82:2-6. Ex. 2 at [0290]; Ex. 1 at 82:23-83:4.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at [0323]; Ex. 1 at 86:14-20.• Ex. 2 at [0296].• Ex. 2 at [0300]. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 3-5; Ex. 39 at 1:1-95.• Ex. 16 at 12.• Ex. 16 at Abstract.• Ex. 39 at 3:4-6.• Ex. 39 at 57-60.• Ex. 39 at 3:70-86. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 18.• Id. at 25.• Id. at 135.• Id. at 147-8. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0049].• Id. at [0052]. <p style="text-align: center;"><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0105].• Id. at [0129].• Id. at [0144].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. • Id. at 3:65-4:2. • Id. at 5:58-6:1. • Id. at 8:38-51. • Id. at 9:35-38. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 33-35. • Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1. • Id. at 88. • Id. at Fig. 6.18. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-60. • Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Chiou (2001)</u></p> <p><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. Ex. 47 at Abstract. Id. at 1:8-11. Id. at 2:21-24. Id. at 2:48-51. Id. at 4:66-5:1. Id. at 6:45-48. Id. at 7:64-8:13. Id. at 8:28-34. Id. at 9:26-28.
controlling flow rates of said aqueous fluid and said carrier fluid to partition the continuously flowing aqueous fluid with the continuously flowing carrier fluid to form a plurality of plugs of the aqueous fluid,	<p><u>Haff (US 6,033,880)</u></p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 9 at 10:23-28. Id. at 10:31-35. Ex. 2 at [0003]; Ex. 1 at 1:14-2:3. Ex. 2 at [0015]; Ex. 1 at 86:21-24. Ex. 2 at [0091]-[0092]; Ex. 1 at 27:22-28:6. Ex. 2 at [0115]; Ex. 1 at 35:11-17. Ex. 2 at [0125]; Ex. 1 at 38:16-19. Ex. 2 at [0287]; Ex. 1 at 82:2-3. Ex. 2 at [0290]; Ex. 1 at 82:23-83:4.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at Figs. 16A, 16B, 17A, 17B, and 21; Ex. 1 at Figs. 16A, 16B, 17A, and 17B. Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12. Ex. 16 at Abstract. Ex. 39 at Fig. 1. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 2. Id. at 17. Id. at 18. Id. at 19. Id. at 62. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0105]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at Fig. 2. Id. at 4:24-30. Id. at 6:60-62. Id. at 6:65-67. Id. at 7:20-21.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Id. at 7:58-62. • Id. at 8:54-55. • Id. at 9:35-38. • Id. at 10:14-19. <p><u>Shaw Stewart (WO 84/02000)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5. • Id. at 12. • Id. at Abstract. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5558.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
each having a substantially uniform size of about 200 μm or less,	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0093]; Ex. 1 at 27:22-28:5. • Ex. 2 at [0117]; Ex. 1 at 36:1-3.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 2 at [0044]. Ex. 2 at [0312]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 58. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4553. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0025].
<p>wherein the target DNA or RNA in said plurality of plugs represents a Poisson distribution,</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0120]; Ex. 1 at 37:12-19. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0162]. <p><u>Lagally (2001)</u></p> <ul style="list-style-type: none"> • Ex. 18 at Abstract.
<p>and at least one member of said plurality comprises a single target DNA or RNA molecule and at least one of the other molecules that can react with the target DNA or RNA molecule; and</p>	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 10:23-25. • Id. at 1:21-27. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at [0012]; Ex. 1 at 5:17-23.• Ex. 2 at [0015]; Ex. 1 at 6:29-7:5.• Ex. 2 at [0020]; Ex. 1 at 8:20-23.• Ex. 2 at [0021]; Ex. 1 at 9:3-7.• Ex. 2 at [0022]; Ex. 1 at 9:20-23.• Ex. 2 at [0058]; Ex. 1 at 15:18-19.• Ex. 2 at [0080]; Ex. 1 at 24:12-13.• Ex. 2 at [0106]; Ex. 1 at 32:14-16.• Ex. 2 at [0120]; Ex. 1 at 37:12-19.• Ex. 2 at [0170]; Ex. 1 at 51:19-52:5.• Ex. 2 at [0316]; Ex. 1 at 84:28-85:10. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 39 at 3:82-86. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 135.• Id. at 147-8. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0012].• Id. at [0013].• Id. at [0014].• Id. at [0049].• Id. at [0052].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none">• Ex. 5 at [0129].• Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at Abstract.• Id. at Field of the invention.• Id. at 3:20-40.• Id. at 7:55-68.• Id. at Fig. 1.• Id. at 4:24-52.• Id. at 3:52-55.• Id. at 3:65-4:2.• Id. at 5:22-25.• Id. at 9:11-17.• Id. at 9:57-61.• Id. at 10:14-19. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 33-35.• Id. at Fig. 6-1. <p><u>Lagally (2001)</u></p> <ul style="list-style-type: none">• Ex. 18 at Abstract. <p><u>Man (2001)</u></p>

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1. <p style="text-align: center;"><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-60. • Id. at Fig. 1. <p style="text-align: center;"><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> • Ex. 44 at Fig. 1, step 1. • Id. at 4:3-5. • Id. at 4:26-28. • Id. at 5:40-44. • Id. at 6:3-6. • Id. at 7:64-8:13. • Id. at 8:15-19. • Id. at 8:28-34. • Id. at 9:26-28.
<p>providing conditions suitable for a polymerase-chain reaction in at least one plug of the plurality of plugs such that the target DNA or RNA is amplified.</p>	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. • Id. at 9:55-10:6. • Id. at 10:15-19. <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p>

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 39 at 2:44-50. Ex. 39 at 3:57-60. Ex. 39 at 3:70-73. <p style="text-align: center;"><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 147-8. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> Ex. 3 at [0012]. Id. at [0013]. Id. at [0014]. <p style="text-align: center;"><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 3:20-40. Id. at 7:55-68. Id. at Fig. 1. Id. at 4:24-52. Id. at Abstract. Id. at Field of the invention. Id. at 6:32-43. Id. at 6:44-50. Id. at 4:62-65. Id. at 5:42-44. Id. at 8:1-11. Id. at 9:19-34. <p style="text-align: center;"><u>Curcio (2002)</u></p>

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 33 at 33-35. Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 89. Id. at 93. Id. at 97-100. Id. at 104. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-60. Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> Ex. 26 at 652. Id. at 655.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 1	Prior Art
	<p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. <p><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> Ex. 47 at Fig. 1, step 1. Id. at 4:3-5. Id. at 4:26-28. Id. at 5:40-44. Id. at 6:3-6. Id. at 7:64-8:13. Id. at 8:15-19. Id. at 8:28-34. Id. at 9:26-28.

Claim 2	Prior Art
The method of claim 1, wherein the step of providing conditions includes heating.	<p><u>Haff (US 6,033,880)</u></p> <p>See claim 1, above.</p> <ul style="list-style-type: none"> Ex. 9 at 1:61-64. Id. at 9:61-10:6. Id. at 10:15-19. Id. at 10:48-51.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 2	Prior Art
	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0080]; Ex. 1 at 24:12-13. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0013].• Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at Abstract.• Id. at Field of the invention.• Id. at 4:24-52.• Id. at 3:26-32.• Id. at 6:32-43.• Id. at 6:44-50.• Id. at 4:62-65.• Id. at 5:42-44.• Id. at 8:1-11.• Id. at 9:19-34. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 33-35.• Id. at Fig. 6-1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none">• Ex. 8 at 97-100.• Id. at 104.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 2	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 5; Ex 39 at 2:44-52. Ex. 39 at 3:57-60. Ex. 39 at 3:70-73. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 147-8. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-60. Id. at Fig. 1. <p><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> Ex. 47 at 8:21-24. Id. at 8:40-44. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0126].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 3	Prior Art
<p>The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor the target DNA or RNA molecule in the at least one member of the plurality of plugs during and/or after the reaction has occurred.</p>	<p>See claim 1, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 8:25-27. • Id. at 25:34-40. • Id. at 25:41-44. • Id. at Fig. 30. • Id. at 26:1-6. • Id. at 26:23-26. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0016]; Ex. 1 at 7:11-14. • Ex. 2 at [0018]; Ex. 1 at 8:3-5. • Ex. 2 at [0020]; Ex. 1 at 8:23-27. • Ex. 2 at [0022]; Ex. 1 at 9:8-12. • Ex. 2 at [0025]; Ex. 1 at 10:4-7. • Ex. 2 at [0054]; Ex. 1 at 14:20-28. • Ex. 2 at [0069]; Ex. 1 at 20:3-5. • Ex. 2 at [0076]; Ex. 1 at 21-26. • Ex. 2 at [0078]; Ex. 1 at 23:12-20. • Ex. 2 at [0086]; Ex. 1 at 25:28-26:1. • Ex. 2 at [0106]; Ex. 1 at 32:6-7. • Ex. 2 at [0110]; Ex. 1 at 34:6-12. • Ex. 2 at [0237]; Ex. 1 at 69:10-11. • Ex. 2 at Figs. 2A and 2B; Ex. 1 at Figs. 2A and 2B.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 3	Prior Art
	<p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 11. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at 4:53-55.• Id. at 6:18-20.• Id. at 8:13-24.• Id. at 8:58-62.• Id. at 10:12-14. <p><u>Man (2001)</u></p> <ul style="list-style-type: none">• Ex. 8 at 100-01.• Id. at Fig. 8.10. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 6-7; Ex. 39 at 2:94-105.• Ex. 39 at 2:59-66.• Ex. 39 at 2:69-70.• Ex. 39 at 2:90-92.• Ex. 39 at 3:57-60. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none">• Ex. 44 at 2.• Id. at 20.• Id. at 28.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 3	Prior Art
	<ul style="list-style-type: none"> • Id. at 61. • Id. at 62. • Id. at 66. • Id. at 68. • Id. at 70. • Id. at 71. • Id. at 73. • Id. at 142. <p style="text-align: center;"><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-60. <p style="text-align: center;"><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0143]. <p style="text-align: center;"><u>Vogelstein (US 6,440,706)</u></p> <ul style="list-style-type: none"> • Ex. 47 at Fig. 1, step 2. • Id. at 2:30-34. • Id. at 2:37-41. • Id. at 2:59-63. • Id. at 3:21-24. • Id. at 5:1-4. • Id. at 5:9-12.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 3	Prior Art
	<ul style="list-style-type: none">• Id. at 7:11-17.• Id. at 7:41-44.• Id. at 8:47-49.• Id. at 9:28.• Id. at 8:47-49.• Id. at 9:34-37.

Claim 6	Prior Art
The method of claim 1, wherein the oil is fluorinated oil.	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0015]; Ex. 1 at 6:21-23.• Ex. 2 at [0094]; Ex. 1 at 28:21-23.• Ex. 2 at [0096]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0117]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p><u>Ramsey (US 6,524,456 B1)</u></p> <ul style="list-style-type: none">• Ex. 14 at 3:63-67.• Id. at 6:36-50. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0047].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 6	Prior Art
	<p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 4; Ex. 39 at 1:39-41. <p><u>Shaw Stewart Thesis</u></p> <ul style="list-style-type: none"> Ex. 44 at 18. Id. at 89. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4164. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at 36. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> Ex. 41 at Abstract. Ex. 41 at 3:40-43. Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23. <p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> Ex. 42 at Abstract. Ex. 42 at 2:29-34. Ex. 42 at 3:16-23.

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 7	Prior Art
The method of claim 1, wherein the carrier fluid further comprises a surfactant.	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0094]; Ex. 1 at 28:21-23.• Ex. 2 at [0096]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0117]; Ex. 1 at 35:28-36:1.• Ex. 2 at [0118]; Ex. 1 at 36:8-10.• Ex. 2 at [0020].• Ex. 2 at [0022].• Ex. 2 at [0041].• Ex. 2 at [0042].• Ex. 2 at [0095].• Ex. 2 at [0300].• Ex. 2 at [0301]. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 4; Ex. 39 at 1:44-48.• Ex. 39 at 2:19-26.• Ex. 39 at 4:26-29. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none">• Ex. 19 at [0041].• Id. at [0075].• Id. at [0076].• Id. at [0077].

APPENDIX H – U.S. Patent No. 8,822,148 (Obviousness)

Claim 8	Prior Art
<p>The method of claim 7, wherein the surfactant is fluorinated surfactant.</p>	<p>See claim 7, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 1:39-41. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> Ex. 29 at Abstract. Id. at 1:13-16. <p><u>Schubert (1993)</u></p> <ul style="list-style-type: none"> Ex. 31 at Abstract. <p><u>Sadtler (1998)</u></p> <ul style="list-style-type: none"> Ex. 34 at Abstract. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> Ex. 41 at Abstract. Ex. 41 at 3:40-43. Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
A microfluidic system comprising:	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at Abstract. <p><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none"> • Ex. 20 at Abstract. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Introduction. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0004]. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
a non-fluorinated microchannel;	<p><u>Quake (US 2002/058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0118]; Ex. 1 at 36:6-10. • Ex. 2 at [0216]; Ex. 1 at 62:27-29. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0040]. <p><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none"> • Ex. 20 at [0027]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Fabrication. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 24.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-57. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 11. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0051].
<p>a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0116]-[0117]; Ex. 1 at 35:25-36:1. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-47. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 6:36-50. • Id. at 3:63-67. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0047]. <p><u>Paolini (US 2002/0131147)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077]. • Id. at [0078]. <p style="text-align: center;"><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none"> • Ex. 20 at [0033]. <p style="text-align: center;"><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p style="text-align: center;"><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at 36. <p style="text-align: center;"><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16. <p style="text-align: center;"><u>Schubert (1994)</u></p> <ul style="list-style-type: none"> • Ex. 31 at Abstract.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<p><u>Sadtler (1998)</u></p> <ul style="list-style-type: none"> Ex. 34 at Abstract. <p><u>Smythe (US 4,253,846)</u></p> <ul style="list-style-type: none"> Ex. 10 at 8:11-16. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> Ex. 41 at Abstract. Ex. 41 at 3:40-43. Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23. <p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> Ex. 42 at Abstract. Ex. 42 at 2:29-34. Ex. 42 at 3:16-23.
<p>at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid,</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at Abstract; Ex. 1 at Abstract. Ex. 2 at [0296]; Ex. 1 at 82:2-3. Ex. 2 at [0092]; Ex. 1 at 27:22-28:6. Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 16 at 3-5; Ex. 39 at 1:1-95. Ex. 16 at 12.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<ul style="list-style-type: none"> Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> Ex. 5 at [0053]. Id. at Fig. 9D. Id. at [0105]. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Abstract. Id. at Experimental. Id. at Fig. 1. Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at Abstract. Id. at Fig. 1. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> Ex. 38 at [0018].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
<p>wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0094]; Ex. 1 at 28:21-25. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-47. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077]. • Id. at [0078]. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 1	Prior Art
	<div> <div> <u>Schubert (1994)</u> <ul style="list-style-type: none"> Ex. 31 at Abstract. </div> <div> <u>Sadtler (1998)</u> <ul style="list-style-type: none"> Ex. 34 at Abstract. </div> <div> <u>Smythe (US 4,253,846)</u> <ul style="list-style-type: none"> Ex. 10 at 2:35-40. Id. at 8:3-16. </div> <div> <u>Smythe (US 3,479,141)</u> <ul style="list-style-type: none"> Ex. 4 at 2:8-20. Id. at 2:35-50. </div> <div> <u>Krafft (U.S. 5,980,936)</u> <ul style="list-style-type: none"> Ex. 41 at Abstract. Ex. 41 at 3:40-43. Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23. </div> </div>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 2	Prior Art
<p>The microfluidic system of claim 1, wherein the at least one plug contains at least one of a cell, a virion, an enzyme, DNA, and RNA.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0018]; Ex. 1 at 7:24-8:3. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:70-86. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. • Id. at 6:60-63. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
Claim 10	Prior Art
<p>The microfluidic system of claim 1, wherein the at least one plug contains at least one reagent for an autocatalytic reaction.</p>	<p>See claim 1, above.</p> <p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. <p><u>Quake (US 2002/058332; US 60/233,037)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 10	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at Abstract; Ex. 1 at Abstract.• Ex. 2 at [0080]; Ex. 1 at 24:12-13.• Ex. 2 at [0002]; Ex. 1 at 1:6-8.• Ex. 2 at [0012]; Ex. 1 at 5:12-13.• Ex. 2 at [0020]; Ex. 1 at 8:17-21.• Ex. 2 at [0021]; Ex. 1 at 9:3-7.• Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 39 at 3:70-86. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0013].• Id. at [0014]. <p style="text-align: center;"><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none">• Ex. 6 at 4:24-52.• Id. at 6:60-63. <p style="text-align: center;"><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at Figure 6-1.• Id. at 33-34. <p style="text-align: center;"><u>Man (2001)</u></p> <ul style="list-style-type: none">• Ex. 8 at 90.• Id. at Table 8.1.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 10	Prior Art
	<p style="text-align: center;"><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
Claim 11	Prior Art
<p>The microfluidic system of claim 10, wherein the autocatalytic reaction is a polymerase-chain reaction.</p>	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <p>See claim 10, above.</p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0002]; Ex. 1 at 1:6-8. • Ex. 2 at [0012]; Ex. 1 at 5:12-13. • Ex. 2 at [0020]; Ex. 1 at 8:17-21. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0013].7 • Id. at [0014].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 11	Prior Art
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 4:24-52. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> Ex. 33 at Figure 6-1. Id. at 33-34. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> Ex. 8 at 90. Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556-59. Id. at Fig. 1. <p><u>Chiou (2001)</u></p> <ul style="list-style-type: none"> Ex. 43 at 2018. Ex. 43 at 2019.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 12	Prior Art
<p>The microfluidic system of claim 1, wherein the volume of the at least one plug is between about two femtoliters and about one hundred nanoliters.</p>	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 2:1-3. • Ex. 2 at [0092]; Ex. 1 at 28:17-19. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Results and Discussion. • Id. at Fig. 1. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 12	Prior Art
	<p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none">• Ex. 38 at [0025].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 13	Prior Art
The microfluidic system of claim 1, wherein the microchannel is made from a polymer, a glass or a metal.	<p>See claim 1, above.</p> <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none">• Ex. 2 at [0088]; Ex. 1 at 27:3-6.• Ex. 2 at [0118]; Ex. 1 at 36:6-10.• Ex. 2 at [0216]; Ex. 1 at 62:27-29. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 7; Ex. 39 at 2:117-122. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Fabrication. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none">• Ex. 36 at 5556-57. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 11. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none">• Ex. 38 at [0051].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
A method of conducting a reaction within at least one plug, comprising the steps of:	<p><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15.
	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0012]; Ex. 1 at 5:20-23.
	<p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0013]. • Id. at [0014].
	<p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0105]. • Id. at [0129].
	<p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at [Abstract]. • Id. at 6:60-63.
	<p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at Figure 6-1. • Id. at 33-34.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> Ex. 17 at Introduction. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> Ex. 7 at 26. Id. at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> Ex. 36 at 5556. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> Ex. 35 at 10-11. Id. at 14. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> Ex. 24 at Abstract. Id. at 289. Id. at 293. <p><u>Tawfik (1998)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0139].
<p>introducing a carrier fluid comprising a fluorinated oil and a fluorinated surfactant comprising a hydrophilic head group in the microchannel into a first non-fluorinated microchannel of a device;</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0015]; Ex. 1 at 7:6-10. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0118]; Ex. 1 at 36:6-10. • Ex. 2 at [0216]; Ex. 1 at 62:27-29. • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:44-47. <p style="text-align: center;"><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none"> • Ex. 14 at 6:36-50. • Id. at 3:63-67. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0047]. <p style="text-align: center;"><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<ul style="list-style-type: none">• Id. at [0076].• Id. at [0077].• Id. at [0078]. <p style="text-align: center;"><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none">• Ex. 20 at [0033]. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none">• Ex. 17 at Experimental. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4163-64. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none">• Ex. 7 at Abstract.• Id. at 25. <p style="text-align: center;"><u>Mason (1997)</u></p> <ul style="list-style-type: none">• Ex. 15 at 4600-01.• Id. at 4604. <p style="text-align: center;"><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 36.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none">• Ex. 29 at Abstract.• Id. at 1:13-16. <p><u>Schubert (1994)</u></p> <ul style="list-style-type: none">• Ex. 31 at Abstract. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none">• Ex. 35 at 10-11. <p><u>Sadtler (1998)</u></p> <ul style="list-style-type: none">• Ex. 34 at Abstract. <p><u>Smythe (US 4,253,846)</u></p> <ul style="list-style-type: none">• Ex. 10 at 8:11-16. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none">• Ex. 41 at Abstract.• Ex. 41 at 3:40-43.• Ex. 41 at 3:47-50.• Ex. 41 at 7:57-8:6.• Ex. 41 at 9:24-51.• Ex. 41 at 11:5-23.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> • Ex. 42 at Abstract. • Ex. 42 at 2:29-34. • Ex. 42 at 3:16-23.
<p>introducing at least one stream of plug-fluid into a first inlet in fluid communication with the first microchannel so that at least one plug forms in the carrier-fluid after the at least one stream contacts the carrier-fluid;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:14-2:3. • Ex. 2 at [0296]; Ex. 1 at 82:2-3. • Ex. 2 at [0015]; Ex. 1 at 7:7-10. • Ex. 2 at [0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at Fig. 16A; Ex. 1 at Fig. 16A. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95.. • Id. at 12. • Id. at Abstract. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id at Experimental. • Id. at Fig. 1. • Id. at Results and Discussion. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p><u>Nisisako (2002)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
<p>wherein: the at least one plug-fluid comprises an aqueous fluid and at least one reagent for an autocatalytic reaction;</p>	<p style="text-align: center;"><u>Haff (US 6,033,880)</u></p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. • Id. at 10:20-28. • Id. at 1:21-27. <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0003]; Ex. 1 at 1:14-17. • Ex. 2 at [0014]; Ex. 1 at 6:3-5. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0020]; Ex. 1 at 8:14-21. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 39 at 3:70-86.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Brown (US 2002/00164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0013]. • Id. at [0014]. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0129]. • Id. at [0144]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. • Id. at 6:60-63. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at Figure 6-1. • Id. at 33-34. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293. <p><u>Tawfik (1998)</u></p> <ul style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0145].
<p>the at least one plug-fluid is immiscible with the carrier-fluid;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0296]; Ex. 1 at 82:2-3. • Ex. 2 at [0116]; Ex. 1 at 35:23-25. • Ex. 2 at [0020]; Ex. 1 at 8:14-16. • Ex. 2 at [0113]; Ex. 1 at 34:26-29. • Ex. 2 at [0125]; Ex. 1 at 38:16-19. • Ex. 2 at [0290]; Ex. 1 at 82:23-83:4. • Ex. 2 at [0287]; Ex. 1 at 82:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
each plug is substantially surrounded on all sides by carrier-fluid;	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0100]; Ex. 1 at 30:2-3. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Id. at 12. • Id. at Abstract. <p style="text-align: center;"><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0105]. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<ul style="list-style-type: none"> • Id. at Results and Discussion. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
<p>and the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0116]-[0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0094]; Ex. 1 at 28:21-25. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p style="text-align: center;"><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<ul style="list-style-type: none">• Id. at [0078]. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none">• Ex. 15 at 4600-01.• Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none">• Ex. 13 at 4164. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none">• Ex. 29 at Abstract.• Id. at 1:13-16. <p><u>Schubert (1994)</u></p> <ul style="list-style-type: none">• Ex. 31 at Abstract. <p><u>Sadtler (1998)</u></p> <ul style="list-style-type: none">• Ex. 34 at Abstract. <p><u>Smythe (US 4,253,846)</u></p> <ul style="list-style-type: none">• Ex. 10 at 2:35-40.• Id. at 8:3-16. <p><u>Smythe (US 3,479,141)</u></p> <ul style="list-style-type: none">• Ex. 4 at 2:8-20.• Id. at 2:35-50.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 20	Prior Art
	<p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23.
Claim 21	Prior Art
<p>The method of claim 20, wherein the autocatalytic reaction is a polymerase-chain reaction.</p>	<p><u>Haff (US 6,033,880)</u></p> <p>See claim 20, above.</p> <ul style="list-style-type: none"> • Ex. 9 at 1:12-15. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0080]; Ex. 1 at 24:12-13. • Ex. 2 at [0002]; Ex. 1 at 1:6-8. • Ex. 2 at [0012]; Ex. 1 at 5:12-13. • Ex. 2 at [0020]; Ex. 1 at 8:17-21. • Ex. 2 at [0021]; Ex. 1 at 9:3-7. • Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0013].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 21	Prior Art
	<ul style="list-style-type: none"> • Id. at [0014]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 4:24-52. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none"> • Ex. 33 at Figure 6-1. • Id. at 33-34. <p><u>Man (2001)</u></p> <ul style="list-style-type: none"> • Ex. 8 at 90. • Id. at Table 8.1. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-59. • Id. at Fig. 1. <p><u>Ghadessy (2001)</u></p> <ul style="list-style-type: none"> • Ex. 27 at 4552-53. <p><u>Katsura (2001)</u></p> <ul style="list-style-type: none"> • Ex. 24 at Abstract. • Id. at 289. • Id. at 293.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 21	Prior Art
	<p data-bbox="280 657 313 848"><u>Tawfik (1998)</u></p> <ul data-bbox="350 1031 418 1257" style="list-style-type: none"> • Ex. 26 at 652. • Id. at 655. <p data-bbox="456 663 488 842"><u>Chiou (2001)</u></p> <ul data-bbox="526 1016 594 1257" style="list-style-type: none"> • Ex. 43 at 2018. • Ex. 43 at 2019.
Claim 22	Prior Art
<p data-bbox="745 1446 850 1923">The method of claim 20, wherein the carrier-fluid comprises a fluorinated compound.</p>	<p data-bbox="745 1058 777 1320">See claim 20, above.</p> <p data-bbox="815 470 847 1037"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul data-bbox="885 653 995 1257" style="list-style-type: none"> • Ex. 2 at [0014]; Ex. 1 at 6:14-16. • Ex. 2 at [0116]-[0117]; Ex. 1 at 32:25-36:1. • Ex. 2 at [0096]. <p data-bbox="1032 449 1065 1058"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul data-bbox="1102 869 1135 1257" style="list-style-type: none"> • Ex. 16 at 4; Ex. 39 at 1:41. <p data-bbox="1172 596 1205 911"><u>Ramsey (US 6,524,456)</u></p> <ul data-bbox="1242 984 1310 1257" style="list-style-type: none"> • Ex. 14 at 3:63-67. • Id. at 6:36-50. <p data-bbox="1347 575 1380 932"><u>Brown (US 2002/0164820)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 22	Prior Art
	<ul style="list-style-type: none">• Ex. 3 at [0047].• Ex. 33 at 36.• Ex. 16 at 4.• Ex. 13 at 4164.• Ex. 19 at [0075].• Id. at [0076].• Id. at [0077].• Id. at [0078].• Ex. 20 at [0033].• Ex. 10 at 8:11-16.• Ex. 41 at Abstract.• Ex. 41 at 3:40-43. <p><u>Curcio (2002)</u></p> <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <p><u>Thorsen (2001)</u></p> <p><u>Paolini (US 2002/0131147)</u></p> <p><u>Shenderov (US 2002/0043463)</u></p> <p><u>Smythe (US 4,253,846)</u></p> <p><u>Krafft (U.S. 5,980,936)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 22	Prior Art
	<ul style="list-style-type: none"> Ex. 41 at 3:47-50. Ex. 41 at 7:57-8:6. Ex. 41 at 9:24-51. Ex. 41 at 11:5-23. <p style="text-align: center;"><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> Ex. 42 at Abstract. Ex. 42 at 2:29-34. Ex. 42 at 3:16-23.
Claim 26	Prior Art
<p>The method of claim 20, wherein the at least one plug contains at least one of a cell, a virion, an enzyme, DNA and RNA.</p>	<p>See claim 20, above.</p> <p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> Ex. 2 at [0018]; Ex. 1 at 7:24-8:3. Ex. 2 at [0021]; Ex. 1 at 9:3-7. Ex. 2 at [0052]; Ex. 1 at 13:23-29. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> Ex. 39 at 3:70-86. <p style="text-align: center;"><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> Ex. 6 at 4:24-52. Id. at 6:60-63.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 26	Prior Art
	<ul style="list-style-type: none">• Ex. 27 at 4552-53.• Ex. 24 at Abstract.• Id. at 289.• Id. at 293.• Ex. 26 at 652.• Id. at 655.• Ex. 38 at [0018]. <p><u>Ghadessy (2001)</u></p> <p><u>Katsura (2001)</u></p> <p><u>Tawfik (1998)</u></p> <p><u>Seki (US 2002/0195463)</u></p>

Claim 31	Prior Art
A microfluidic system comprising:	<ul style="list-style-type: none">• Ex. 2 at Abstract; Ex. 1 at Abstract.• Ex. 3 at Abstract.• Ex. 19 at Abstract. <p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <p><u>Brown (US 2002/0164820)</u></p> <p><u>Paolini (US 2002/0131147)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<p><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none"> • Ex. 20 at Abstract. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Introduction. <p><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 26. • Id. at Abstract. <p><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556. <p><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0004]. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
a non-fluorinated microchannel;	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0118]; Ex. 1 at 36:6-10.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none"> • Ex. 2 at [0216]; Ex. 1 at 62:27-29. <p style="text-align: center;"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. <p style="text-align: center;"><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none"> • Ex. 3 at [0040]. <p style="text-align: center;"><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none"> • Ex. 20 at [0027]. <p style="text-align: center;"><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Fabrication. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at 24. <p style="text-align: center;"><u>Burns (1996)</u></p> <ul style="list-style-type: none"> • Ex. 36 at 5556-57. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 11.
a fluorinated carrier fluid;	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0003]; Ex. 1 at 1:14-17.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none">• Ex. 2 at [0014]; Ex. 1 at 6:14-16.• Ex. 2 at [0116]-[0117]; Ex. 1 at 35:28-36:1. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none">• Ex. 16 at 4; Ex. 39 at 1:44-47. <p><u>Brown (US 2002/0164820)</u></p> <ul style="list-style-type: none">• Ex. 3 at [0047]. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none">• Ex. 19 at [0075].• Id. at [0076].• Id. at [0077].• Id. at [0078]. <p><u>Shenderov (US 2002/0043463)</u></p> <ul style="list-style-type: none">• Ex. 20 at [0033]. <p><u>Ramsey (US 6,524,456)</u></p> <ul style="list-style-type: none">• Ex. 14 at 6:36-50.• Id. at 3:63-67. <p><u>Curcio (2002)</u></p> <ul style="list-style-type: none">• Ex. 33 at 36. <p><u>Smythe (US 4,253,846)</u></p>

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none"> • Ex. 10 at 8:11-16. <p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6. • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23. <p><u>Parris (U.S. 5,739,036)</u></p> <ul style="list-style-type: none"> • Ex. 42 at Abstract. • Ex. 42 at 2:29-34. • Ex. 42 at 3:16-23. <p><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0051].
<p>a fluorinated surfactant comprising a hydrophilic head group in the carrier fluid;</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0094]; Ex. 1 at 28:21-25. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077]. • Id. at [0078].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<p data-bbox="280 653 313 840"><u>Mason (1997)</u></p> <ul data-bbox="350 968 418 1253" style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p data-bbox="456 443 488 1050"><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul data-bbox="526 1058 558 1253" style="list-style-type: none"> • Ex. 16 at 4. <p data-bbox="596 642 628 852"><u>Thorsen (2001)</u></p> <ul data-bbox="665 1010 698 1253" style="list-style-type: none"> • Ex. 13 at 4164. <p data-bbox="735 579 768 915"><u>Delpuech (US 5,185,099)</u></p> <ul data-bbox="805 968 873 1253" style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16. <p data-bbox="911 638 943 856"><u>Schubert (1994)</u></p> <ul data-bbox="980 968 1013 1253" style="list-style-type: none"> • Ex. 31 at Abstract. <p data-bbox="1050 653 1083 844"><u>Sadtler (1998)</u></p> <ul data-bbox="1120 968 1153 1253" style="list-style-type: none"> • Ex. 34 at Abstract. <p data-bbox="1174 600 1206 907"><u>Krafft (U.S. 5,980,936)</u></p> <ul data-bbox="1243 957 1393 1253" style="list-style-type: none"> • Ex. 41 at Abstract. • Ex. 41 at 3:40-43. • Ex. 41 at 3:47-50. • Ex. 41 at 7:57-8:6.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none"> • Ex. 41 at 9:24-51. • Ex. 41 at 11:5-23.
<p>and at least one plug comprising an aqueous plug-fluid in the microchannel and substantially encased by the carrier-fluid,</p>	<p><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at Abstract; Ex. 1 at Abstract. • Ex. 2 at [0296]; Ex. 1 at 82:2-3. • Ex. 2 at [0092]; Ex. 1 at 27:22-28:6. • Ex. 2 at [100]; Ex. 1 at 30:2-3. <p><u>Shaw Stewart (WO 84/02000; GB 2,097,692)</u></p> <ul style="list-style-type: none"> • Ex. 16 at 3-5; Ex. 39 at 1:1-95. • Ex. 16 at 12. • Ex. 16 at Abstract. <p><u>Bohm (US 2003/0007898)</u></p> <ul style="list-style-type: none"> • Ex. 5 at [0053]. • Id. at Fig. 9D. • Id. at [0105]. <p><u>Corbett (US 5,270,183)</u></p> <ul style="list-style-type: none"> • Ex. 6 at 6:60-63. <p><u>Nisisako Abstract (2001)</u></p> <ul style="list-style-type: none"> • Ex. 17 at Abstract. • Id. at Experimental. • Id. at Fig. 1.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none"> • Id. at Results and Discussion. <p style="text-align: center;"><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4163. <p style="text-align: center;"><u>Nisisako (2002)</u></p> <ul style="list-style-type: none"> • Ex. 7 at Abstract. • Id. at Fig. 1. <p style="text-align: center;"><u>Burns (2001)</u></p> <ul style="list-style-type: none"> • Ex. 35 at 10-11. • Id. at 14. <p style="text-align: center;"><u>Seki (US 2002/0195463)</u></p> <ul style="list-style-type: none"> • Ex. 38 at [0018].
<p>wherein the fluorinated surfactant is present at a concentration such that surface tension at the plug-fluid/microchannel wall interface is higher than surface tension at the plug-fluid/carrier fluid interface.</p>	<p style="text-align: center;"><u>Quake (US 2002/0058332; US 60/233,037)</u></p> <ul style="list-style-type: none"> • Ex. 2 at [0117]; Ex. 1 at 35:28-36:1. • Ex. 2 at [0094]; Ex. 1 at 28:21-25. • Ex. 2 at [0118]; Ex. 1 at 36:8-10. <p style="text-align: center;"><u>Paolini (US 2002/0131147)</u></p> <ul style="list-style-type: none"> • Ex. 19 at [0041]. • Id. at [0075]. • Id. at [0076]. • Id. at [0077].

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<ul style="list-style-type: none"> • Id. at [0078]. <p><u>Mason (1997)</u></p> <ul style="list-style-type: none"> • Ex. 15 at 4600-01. • Id. at 4604. <p><u>Thorsen (2001)</u></p> <ul style="list-style-type: none"> • Ex. 13 at 4164. <p><u>Delpuech (US 5,185,099)</u></p> <ul style="list-style-type: none"> • Ex. 29 at Abstract. • Id. at 1:13-16. <p><u>Schubert (1994)</u></p> <ul style="list-style-type: none"> • Ex. 31 at Abstract. <p><u>Sadtler (1998)</u></p> <ul style="list-style-type: none"> • Ex. 34 at Abstract. <p><u>Smythe (US 4,253,846)</u></p> <ul style="list-style-type: none"> • Ex. 10 at 2:35-40. • Id. at 8:3-16. <p><u>Smythe (US 3,479,141)</u></p> <ul style="list-style-type: none"> • Ex. 4 at 2:8-20. • Id. at 2:35-50.

APPENDIX I – U.S. Patent No. 8,889,083 (Obviousness)

Claim 31	Prior Art
	<p><u>Krafft (U.S. 5,980,936)</u></p> <ul style="list-style-type: none">• Ex. 41 at Abstract.• Ex. 41 at 3:40-43.• Ex. 41 at 3:47-50.• Ex. 41 at 7:57-8:6.• Ex. 41 at 9:24-51.• Ex. 41 at 11:5-23.

EXHIBIT G

REDACTED

EXHIBIT H

Walter, Derek

From: Drake, Lauren <LDrake@irell.com>
Sent: Tuesday, August 29, 2017 7:24 PM
To: Walter, Derek; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; Reines, Edward; RainDance 10X Service
Cc: #10X/RainDance [Int]; Cottrell@rlf.com; rawnsley@rlf.com; Pedi, Nicole K. <Pedi@rlf.com> (Pedi@rlf.com)
Subject: RE: Bio-Rad/10X - Opening Report of Professor Chang

Derek:

We disagree with your suggestion that the length of Professor Chang's report somehow necessitates additional deposition time. The length of Professor Chang's report is the result (at least in part) of Plaintiffs' failure to meaningfully narrow the number of asserted claims. We will not provide additional dates for continued deposition.

We will respond as soon as possible regarding Dr. Sia's deposition. In the meantime, please immediately provide Mr. Malackowski's and Dr. Shinoff's availability.

Best,

Lauren

Lauren Drake | Irell & Manella LLP

1800 Avenue of the Stars, Suite 900 | Los Angeles, CA 90067

Direct | 310.203.7043 Fax | 310.203.7199

From: Walter, Derek [<mailto:Derek.Walter@weil.com>]
Sent: Tuesday, August 29, 2017 6:28 PM
To: Drake, Lauren; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; ~Reines, Edward; RainDance 10X Service
Cc: #10X/RainDance [Int]; ~Cottrell, Fred; rawnsley@rlf.com; Pedi, Nicole K. <Pedi@rlf.com> (Pedi@rlf.com)
Subject: RE: Bio-Rad/10X - Opening Report of Professor Chang

Lauren:

Following up on the email below, please let us know as soon as you can regarding the date for Dr. Sia's deposition. His schedule is filling up quickly, and we would like to lock down the date. We would prefer to proceed on the 20th. Please let us know if that works. We've also offered the 17th, but we would in fact prefer the 16th.

Thanks,

Derek

From: Walter, Derek
Sent: Monday, August 28, 2017 6:39 PM
To: Drake, Lauren; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; Reines, Edward; RainDance 10X Service
Cc: #10X/RainDance [Int]; Cottrell@rlf.com; rawnsley@rlf.com; Pedi, Nicole K. <Pedi@rlf.com> (Pedi@rlf.com)
Subject: RE: Bio-Rad/10X - Opening Report of Professor Chang

Lauren:

We'll proceed with Dr. Chang's deposition on October 13. However, we note that Dr. Chang's report is roughly 1,700 pages. Given the length of this report, we think additional deposition time beyond the normal 7 hours is warranted. Please provide additional dates for continued deposition.

Also, please promptly confirm one of the proposed dates for Dr. Sia's deposition.

Thanks,

Derek

From: Drake, Lauren [<mailto:LDrake@irell.com>]

Sent: Monday, August 21, 2017 2:49 PM

To: bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; Walter, Derek; Reines, Edward; RainDance 10X Service

Cc: #10X/RainDance [Int]; Cottrell@rlf.com; rawnsley@rlf.com; Pedi, Nicole K. <Pedi@rlf.com> (Pedi@rlf.com)

Subject: Bio-Rad/10X - Opening Report of Professor Chang

Counsel:

Please see attached. Professor Chang is available for deposition on October 13, 2017.

Best,

Lauren

Lauren Drake | Irell & Manella LLP

1800 Avenue of the Stars, Suite 900 | Los Angeles, CA 90067

Direct | 310.203.7043 Fax | 310.203.7199

PLEASE NOTE: This message, including any attachments, may include privileged, confidential and/or inside information. Any distribution or use of this communication by anyone other than the intended recipient(s) is strictly prohibited and may be unlawful. If you are not the intended recipient, please notify the sender by replying to this message and then delete it from your system. Thank you.

The information contained in this email message is intended only for use of the individual or entity named above. If the reader of this message is not the intended recipient, or the employee or agent responsible to deliver it to the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited. If you have received this communication in error, please immediately notify us by email, postmaster@weil.com, and destroy the original message. Thank you.

EXHIBIT I

Walter, Derek

From: Tuan, Elizabeth <ETuan@irell.com>
Sent: Friday, August 04, 2017 9:15 AM
To: RainDance 10X Service; mfarnan@farnanlaw.com; bfarnan@farnanlaw.com
Cc: #10X/RainDance [Int]; Cottrell@rlf.com; Rawnsley@RLF.com
Subject: 10X/Bio-Rad/Chicago - Huck Disclosure
Attachments: Huck PO Acknowledgment.pdf; Huck CV - 2017.pdf

Counsel:

Pursuant to Paragraph 9 of the Protective Order, 10X discloses Prof. Wilhelm T. S. Huck.

Attached please find Prof. Huck's signed Acknowledgment of Protective Order and his CV (which contains information relating to his present and past employment).

Prof. Huck has previously testified at deposition in the following cases, in each instance on behalf of 10X Genomics, Inc.:

- 10X Genomics, Inc. v. Univ. of Chicago, IPR2015-01157
- 10X Genomics, Inc. v. Univ. of Chicago, IPR2015-01162
- 10X Genomics, Inc. v. Univ. of Chicago, IPR2015-01558

As Prof. Huck's CV indicates, in the past five years, he has consulted with Sphere Fluidics and Cytofind Diagnostics. His work with Sphere Fluidics involves microfluidics, including the use of surfactants for droplet-based microfluidics. His work with Cytofind Diagnostics involves the use of droplet-based microfluidic technology to detect circulating tumor cells. Neither project involves DNA or RNA sequencing tools; instead, their focus is on selecting individual cells based on secreted molecules.

Aside from having previously consulted for 10X, Prof. Huck does not have any relationship (previous or current) with the parties.

Thank you,
Elizabeth

Elizabeth Tuan | Associate
Irell & Manella LLP
1800 Avenue of the Stars | Los Angeles, CA 90067
Direct: (310) 203-7672 | Fax: (310) 203-7199
etuan@irell.com | www.irell.com

PLEASE NOTE: This message, including any attachments, may include privileged, confidential and/or inside information. Any distribution or use of this communication by anyone other than the intended recipient(s) is strictly prohibited and may be unlawful. If you are not the intended recipient, please notify the sender by replying to this message and then delete it from your system. Thank you.

EXHIBIT J

Prof. dr. Wilhelm T.S. Huck
Institute for Molecules and Materials
Radboud University
Heyendaalseweg 135
NL-6525 AJ Nijmegen, The Netherlands
Phone: +31 24 3652138
e-mail: w.huck@science.ru.nl
website: <http://www.ru.nl/physicalorganicchemistry>

Positions and Employment

2010- Professor of Physical Organic Chemistry, Institute for Molecules and Materials,
Radboud University
2007-2012 Professor of Macromolecular Chemistry, University of Cambridge
2004-2010 Director, Melville Laboratory for Polymer Synthesis, University of Cambridge
2003-2007 Reader in Chemistry, University of Cambridge
1999-2009 College Lecturer and Fellow of Gonville and Caius College
1999-2003 University Lecturer. University of Cambridge, UK

2010 Co-founder. Sphere Fluidics, Cambridge, UK
2015 Co-founder. Cytofind Diagnostics, Nijmegen.

Memberships of Boards and Editorships

2017- Board Member of the Australian Institute for BioNanotechnology, University of
Queensland
2015- Vice-chair of Scientific Advisory Board MaxSynBio, Germany
2014 Editorial Executive Board of *Advanced Science* (Wiley)
2007-2010 Chairman of the Editorial Board of *Chemical Society Reviews* (RSC)
2005-2010 Editorial Advisory Board of *Soft Matter* (RSC)
2004- International Advisory Board *Advanced Functional Materials*

Honors

2017 Co-PI on 18.8 M€ Gravitation Grant ' Building a Synthetic Cell'.
2016 Spinoza Prize (highest academic honor in the Netherlands)
2015 Elected vice-chair of the Scientific Advisory Board of MaxSynBio
2012 Elected to the Royal Netherlands Academy of Arts and Sciences (KNAW)
2011 NWO-Vici award
2010 ERC Advanced Grant
2009 Friedrich Wilhelm Bessel Research Award of the Humboldt Foundation
2004 DuPont Young Professor Award
2001 Young Investigator Medal of the Royal Society of Chemistry, MacroGroup UK
1997 DSM Award for best Ph.D. research in the Netherlands in polymer-related chemistry

1996 Schloßmann Award from the Max Planck Gesellschaft zur Förderung der Wissenschaften

Consultancy and other activities

2017- Scientific Advisory Board Sphere Fluidics, Cambridge, UK.

2015 Co-founder Cytofind Diagnostics

2010-2016 Board of Directors Sphere Fluidics

2010 Co-founder Sphere Fluidics

EXHIBIT K

**UNITED STATES DISTRICT COURT
DISTRICT OF DELAWARE**

BIO-RAD LABORATORIES, INC.

and

THE UNIVERSITY OF CHICAGO,

Plaintiffs,

v.

10X GENOMICS, INC.,

Defendant.

C.A. No. 15-152-RGA

NON-DISCLOSURE & LIMITED USE AGREEMENT

It is acknowledged and understood that:

- On June 29, 2017, defendant 10X Genomics, Inc. (“Discloser”) initially provided for analysis and/or testing certain materials (the “Materials”) to plaintiffs Bio-Rad Laboratories, Inc. (the “Receiver”) and The University of Chicago that did not bear confidentiality designations pursuant to the Stipulated Protective Order in this Litigation (dated June 27, 2016) (the “Protective Order”);
- After Receiver commenced its analysis and/or testing on the Materials, Discloser designated the Materials as “Outside Attorneys’ Eyes Only Information” (“OAEO”) under the Protective Order, therefore, preventing Receiver from completing its analysis and/or testing of the Materials;
- The Materials have a limited “shelf-life,” and after Receiver’s analysis and/or testing ceased, the Materials expired and became no longer usable.

WHEREFORE, subject to execution of this Agreement, Discloser intends to provide another set of Materials to Receiver for analysis and/or testing thereon, including:

“Materials”	
<u>Item</u>	<u>Description</u>
120207	GemCode™ Gel Bead & Library kit, 16 rxns (120205, 120203)
120204	GemCode™ Chip Kit, 48 rxns
120229	Chromium™ Genome Library, Gel Bead & Multiplex Kit, 16 rxns (120215, 120214, 120262)
120216	Chromium™ Genome Chip Kit, 48 rxns
120258	Chromium™ Genome Library & Gel Bead Kit v2, 16 rxns (120255, 120214)
120257	Chromium™ Genome Chip Kit v2, 48 rxns
120233	Chromium™ Single Cell 3' Library, Gel Bead, & Multiplex Kit, 16 rxns (120230, 120231, 120262)
120232	Chromium™ Single Cell 3' Chip Kit, 48 rxns
120267	Chromium™ Single Cell 3' Library & Gel Bead Kit v2, 4 rxns (120264, 120265)
1000009	Chromium™ Single Cell A Chip Kit, 16 rxns

1. Purpose. The purpose of this Agreement is to protect Discloser’s confidential information related to the Materials going forward, and to preserve any confidentiality necessary under patent and/or trade secret laws.

2. Definition of Confidential Information. For purposes of this Agreement, “Confidential Information” shall include all non-public information that is not reasonably ascertainable from public sources which Receiver may acquire from analysis and/or testing of the Materials provided by Discloser.

3. Receiver’s Employees’ Obligations. Receiver’s undersigned employee acknowledges and agrees: (a) to hold and maintain the Confidential Information in confidence, and shall not disclose any such Confidential Information to any other party, except for: (i) any party that has duly executed this Agreement, (ii) Weil, Gotshal, & Manges LLP, Receiver’s Litigation counsel, (iii) any expert witness whom has duly executed the Acknowledgement of Protective Order in this Litigation, and/or (iv) when

required to do so by law; and (b) that all Confidential Information shall only be used for the purposes of this Litigation.

4. Receiver's Undersigned Employee's Freedom to Perform Work Duties. Nothing in this Agreement shall prevent Receiver's undersigned employee from performing their regular duties for Receiver as long as s/he does not use any Confidential Information.

5. Waiver of Claims From Discloser's Earlier Failure to Designate Materials. Discloser waives any claims against Receiver and/or its employees regarding Receiver's and/or its employees' analysis and/or use of the Materials before Discloser designated the Materials as OAEO under the Protective Order.

6. Integration / Modification. This Agreement states the entire agreement between Discloser and Receiver concerning the disclosure of the Materials. Any addition or modification to this Agreement must be made in writing and signed by the Discloser and Receiver.

7. Severability. If any of the provisions of this Agreement are found to be unenforceable, the remainder shall be enforced as fully as possible and the unenforceable provision(s) shall be deemed modified to the limited extent required to permit enforcement of the Agreement as a whole.

WHEREFORE, the undersigned acknowledges that s/he has read and understands this Agreement, and voluntarily accepts the duties and obligations set forth herein.

DATED this _____ day of _____, 20_____.

Signature

Printed Name

EXHIBIT L

Walter, Derek

From: Drake, Lauren <LDrake@irell.com>
Sent: Thursday, July 20, 2017 7:16 PM
To: Walter, Derek; Rawnsley, Jason J.; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; Reines, Edward; RainDance 10X Service
Cc: Strub, Michael; Courtney, Dennis; Tuan, Elizabeth; Iancu, Andrei; Gindler, David; Cottrell@rlf.com
Subject: RE: Supplemental 4(d) Disclosure (Raindance v. 10X Genomics)

Derek:

We disagree with your contention that our 4(d) disclosures are deficient in any respect. First, 10X first served its 4(d) disclosures on July 12, 2016. 10X supplemented its 4(d) disclosures on June 23, 2017. Plaintiffs waited over a *year* since 10X first served its disclosures and nearly a *month* since 10X served supplemental disclosures to raise their concerns, leaving 10X with only *four days* before the close of fact discovery to potentially further supplement its disclosures. This considerable delay is markedly prejudicial against 10X and for that reason alone, Plaintiffs' concerns are inappropriate.

Beyond Plaintiffs' delay in raising the alleged deficiencies below, Plaintiffs' contentions are wholly unfounded. First, Plaintiffs' statement "10X's contentions fail to preserve any invalidity argument based on obviousness at least because (1) they fail to state which claims 10X is relying upon for its various combinations of art" is nonsensical. 10X provided ample information sufficient to provide Plaintiffs with notice of the invalidity arguments 10X intends to rely upon, including claim charts for all asserted claims with pincites to specific prior art references. There is no threat of prejudice to Plaintiffs. Further, Plaintiffs cite no authority for its assertion that 10X has waived "insufficiently stated invalidity theor[ies]."

10X reserves all rights.

Best,

Lauren

Lauren Drake | Irell & Manella LLP

1800 Avenue of the Stars, Suite 900 | Los Angeles, CA 90067

Direct | 310.203.7043 Fax | 310.203.7199

From: Walter, Derek [mailto:Derek.Walter@weil.com]
Sent: Monday, July 17, 2017 3:37 PM
To: Rawnsley, Jason J.; bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; ~Reines, Edward; RainDance 10X Service
Cc: Strub, Michael; Courtney, Dennis; Tuan, Elizabeth; Iancu, Andrei; Gindler, David; ~Cottrell, Fred; Drake, Lauren
Subject: RE: Supplemental 4(d) Disclosure (Raindance v. 10X Genomics)

Counsel:

We've reviewed these supplemental disclosures carefully, and they are deficient in at least the following respects:

- 10X's contentions fail to preserve any invalidity argument based on obviousness at least because (1) they fail to state which claims 10X is relying upon for its various combinations of art and (2) 10X fails to provide any rationale for combining the references listed in 10X's contentions. 10X's contentions are provided mainly in the form of charts that merely identify where certain claim elements are present in various prior art reference. Under *KSR*, this does not suffice. As things stand, Plaintiffs cannot discern how and why 10X is proposing to combine the countless possible combinations of art present in 10X's contentions, and Plaintiffs thus cannot fully discern 10X's obviousness theory.
- 10X's contentions related to enablement and written description are conclusory and thus fail to preserve any invalidity argument based on § 112. Indeed, 10X identifies certain claim elements that are allegedly not adequately described and/or that could not be carried out without undue experimentation, but does not provide any reasoning for why this is supposedly the case.
- Finally, 10X's contentions related to indefiniteness are insufficient because they also offer no reasoning as to why any claim terms are indefinite.

To the extent 10X does not supplement these prior to the close of discovery, we will seek to strike any insufficiently stated invalidity theory 10X later seeks to rely upon.

Thanks,

Derek

From: Rawnsley, Jason J. [<mailto:Rawnsley@RLF.com>]

Sent: Friday, June 23, 2017 3:01 PM

To: bfarnan@farnanlaw.com; mfarnan@farnanlaw.com; Walter, Derek; Reines, Edward; RainDance 10X Service

Cc: Strub, Michael; Courtney, Dennis; Tuan, Elizabeth; Iancu, Andrei; Gindler, David; Cottrell@rlf.com; Drake, Lauren

Subject: Supplemental 4(d) Disclosure (Raindance v. 10X Genomics)

Counsel:

Attached please find 10X Genomics, Inc.'s Supplemental Initial Paragraph 4(d) Disclosure. Owing to size, the exhibits will be sent separately.

Jason J. Rawnsley
Richards, Layton & Finger, P.A.
920 North King Street
Wilmington, DE 19801
(302) 651-7550

The information contained in this electronic communication is intended only for the use of the individual or entity named above and may be privileged and/or confidential. If the reader of this message is not the intended recipient, you are hereby notified that any unauthorized dissemination, distribution or copying of this communication is strictly prohibited by law. If you have received this communication in error, please immediately notify us by return e-mail or telephone (302-651-7700) and destroy the original message. Thank you.

The information contained in this email message is intended only for use of the individual or entity named above. If the reader of this message is not the intended recipient, or the employee or agent responsible to deliver it to the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly

prohibited. If you have received this communication in error, please immediately notify us by email, postmaster@weil.com, and destroy the original message. Thank you.

PLEASE NOTE: This message, including any attachments, may include privileged, confidential and/or inside information. Any distribution or use of this communication by anyone other than the intended recipient(s) is strictly prohibited and may be unlawful. If you are not the intended recipient, please notify the sender by replying to this message and then delete it from your system. Thank you.